MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLV SEPTEMBER-OCTOBER, 1953 No. 5

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[MYCOLOGIA for July–August (45: 481–626) was issued August 24, 1953]

PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

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MYCOLOGIA

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VOL. XLV SEPTEMBER–OCTOBER, 1953 NO. 5

MICROASSAY OF ANTIFUNGAL PROPERTIES OF STEROID HORMONES AND OTHER COMPOUNDS[1]

J. E. TARBET, M. OURA, AND THOMAS H. STERNBERG[2]

(WITH 9 FIGURES)

Oster and Golden (**25**) have stated that assays of fungicides modeled after the "germination tests" employed by plant pathologists would meet some of the needs of pharmacology provided suitable indicator fungi are found among the pathogens of mammals. Species of *Candida* possess certain obvious advantages as indicators of this class. Large numbers of viable spores are regularly obtainable in homogeneous suspension from cultures of *Candida*, and risks of accidental laboratory infection are not great with this organism.

The principal purpose of this study has thus been to apply the enumeration in cultures of the budding cells of a pathogenic strain of *Candida tropicalis* as a measure of the growth of the organism in the presence of various compounds. The effects of a number of steroid hormones and allied substances, as well as of active nonsteroid compounds, on the growth of *C. tropicalis*, *Microsporum fulvum*, *Scopulariopsis brevicaulis* and *Saccharomyces cerevisiae*

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[2] This research was supported in part by a grant-in-aid from Ciba Pharmaceutical Products, Inc., Summit, New Jersey. The authors wish to acknowledge their indebtedness for technical assistance to Mr. Frank Pero.

[MYCOLOGIA for July–August (**45**: 481–626) was issued August 24, 1953]

have been examined. Included are (a) a description and an evaluation of the technic employed, (b) a comparison of the median effective dose values (M)[3] obtained with some of the compounds, and (c) a preliminary investigation of the effects of combinations of active drugs on the growth of *C. tropicalis*.

More information concerning the effect of steroid compounds on the growth of fungi appears to be desirable. A number of investigators (**4**, **17**, **24**, **28**) have noted enhancement of fungus infections in experimental animals when the steroid hormone, cortisone, was administered. Opinion in general appears to be that cortisone and adrenocorticotropic hormone (ACTH) may exert an unfavorable effect in any class of deep infections (**1**, **2**, **3**, **9**, **11**, **18**, **33**, **34**, **35**, **38**, **39**). It has not been suggested that the disease microorganisms are in any way directly affected by these hormones, but that the resistance of the host is impaired.

The influence of the androgens and estrogens in infectious diseases may be of a different order than that of cortisone and ACTH. Frazier *et al.* (**8**), Hudson and associates (**16**), and von Haam and Rosenfeld (**12**) are among those who have noted improvement in systemic bacterial or viral infections when sex hormones were given. Benefits in superficial fungus infections have been noted, sometimes as a side effect of the parenteral administration of the sex hormones for other conditions (**10**, **30**, **32**). The benefits have been ascribed to such changes in the skin, hair and nails as normally occur at puberty, but which may also follow treatment with androgens or estrogens.

Some steroid hormones can affect the growth of fungi directly. Reiss (**29**) was able to induce trichophytosis *rubrum* in the castrated rabbit, and to limit the disease by topical application of sex hormones (**30**). He also demonstrated the *in vitro* fungistasis of *Trichophyton rubrum* (*purpureum*) and *Trichophyton mentagrophytes* by testosterone, estradiol and desoxycorticosterone (**31**). Hruszek (**15**) had shown earlier that anterior pituitary and ovarian follicular preparations will arrest the cultural development of *T. mentagrophytes* and *Microsporum audouini*.

[3] M is defined as the concentration of drug which reduces the number of growing organisms to one-half.

It is also of interest that the inclusion in the culture medium of stilbestrols and other synthetics with physiological activities like those of the sex hormones, has been found to modify the growth of fungi and bacteria (**5**, **13**, **31**). Stilbestrol has also been used successfully to protect mice in streptococcal infections (**6**).

Mammalian sex hormones may affect some fungi in the actual capacity of hormones. Plumb and Durrell (**26**) reported that traces of estrin in the culture medium suppressed the production of gametangia and zygospores in *Rhizopus nigricans* without interfering with vegetative growth.

METHODS AND MATERIALS

Indicator Organisms. A number of "monilia" isolates from different sources were examined in a preliminary survey before choice was settled on an organism obtained from an axillary abscess and identified as *Candida tropicalis* (Castellani) Berkhout.[4] This microorganism was selected for study because of its rapid growth in the medium employed, and its large cell size. *Microsporum fulvum* Sabouraud, *Saccharomyces cerevisiae* Hansen and *Scopulariopsis brevicaulis* (Saccardo) Bainier were also used as indicators in some of the experiments. Stock cultures of the organisms were maintained by a serial transfer on Sabouraud's agar slants in screw-cap culture bottles of the type employed by Wilson and Plunkett (**40**).

Assay Medium. The following broth was employed in all of the experiments:

Dextrose	20 gm
Glycine	2 gm
$MgCl_2 \cdot H_2O$	2 gm
Phosphate buffer[5] pH 6.4	200 ml
Distilled water	800 ml

[4] Intraperitoneal inoculation of mice with this fungus, using eight million cells, resulted after four weeks in spleen, liver and kidney lesions. Application of the procedures for the speciation of *Candida* proposed by Martin *et al.* (**19**) and Martin and Jones (**20**) placed it in the *Candida tropicalis* group producing large gray colonies with a mycelial fringe on blood agar at 37° C, acid and gas in glucose, sucrose and maltose but not in lactose, and developing no chlamydospores on cornmeal agar.

[5] Sorensen phosphate standards. *Practical Physiological Chemistry.* P. B. Hawk and O. Bergeim, 11th Ed., 1944.

Preparation of the Test Compounds.[6] The compounds used in the experiments were weighed analytically. Volumetric technic was employed to obtain the dilutions of the substances required, using the assay medium as diluent. Five-tenths ml volumes of each dilution of each substance, at double the final concentrations desired, were pipetted into as many replicate tubes as required to assemble the experiments. To the tubes was then added 0.5 ml of medium containing known numbers of fungus cells.

All of the test substances were soluble in the medium at the low concentrations (up to about ten parts per million (ppm)). Those substances which were not soluble in the medium at the higher concentrations were first dissolved in a minimum of acetone, the required volume of medium was then added, and the proper volume recovered by evaporation. In most instances this treatment resulted in a clear solution. When it did not (α-estradiol, progesterone, testosterone, testosterone undecylenate), a fine suspension was obtained.

Sterile glassware, instruments and medium were used in the preparation of the test compounds and in assembling the experiments, but subjecting the compounds themselves to the ordinary processes of sterilization was avoided because some of them were

[6] The following crystalline substances were furnished to the authors by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

Cholesterol
Desoxycorticosterone
Desoxycorticosterone glucoside
Diethyl-2-hydroxyethyl-(3-keto-Δ^4-17-pregnadienyl) NH,BR
α-estradiol
Pregnenolone
Progesterone
17-amino-Δ^4-androstene-3-one HCl
17-amino-Δ^4-androstene-3-ol
Testosterone
Testosterone undecylenate
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr
20-amino-3β-hydroxy-5-pregnene HCl
o-dichloracetyloxine
3,4-dichloro-ω-trichloromethyl acrylophenone
Bradosol(R) (phenoxyethyl-dimethyl-dodecyl NH,Br)

Cortisone acetate and hydrocortisone acetate were obtained from Merck and Company, Inc., Rahway, New Jersey.

heat labile. This did not result in contamination, possibly owing to the fact that all of the test compounds were stored in sealed vials for several weeks before use.

Preparation of Inocula. Inocula for the tests were prepared by flooding the surface of 15-day-old stock cultures of the fungi with 6 ml of medium. Pure suspensions of the conidia of *M. fulvum* and *S. brevicaulis* were obtained in this way. The cultures of the yeast-like fungi yielded 80–95% unicellular forms. Five ml of the spore suspensions were transferred to 50 ml flasks containing 25 ml of medium. After thorough shaking, the fungus cells were counted, using the leukocyte chamber of the hemacytometer for the yeast-like cells and a drop of inoculum beneath a coverslip (22 mm², No. 1 thin) for enumerating the conidia of the two mycelial forms. Suitable dilutions of the fungus cell suspensions were then prepared to contain the following numbers of individuals:

Organism	*Numbers of cells in inoculum*
C. tropicalis	200–400 per cmm
M. fulvum	6–10 per field (× 100)
S. cerevisiae	200–400 per cmm
S. brevicaulis	6–10 per field (× 430)

The numbers of cells in the assay cultures at the start of incubation were, of course, half those indicated above, because the inocula prepared as above were further diluted in the test tubes with medium containing suitable concentrations of the test compounds. These numbers of cells gave rise to cultures of a density convenient for enumeration after convenient intervals of incubation (6–7 hours for *S. brevicaulis;* 13–16 hours for *C. tropicalis;* 17–24 hours for *M. fulvum* and *S. cerevisiae*).

Growth and germination curves for the microorganisms were obtained in a preliminary experiment in order to ascertain the most satisfactory intervals of incubation. Many replicate cultures of each organism were prepared in one ml volumes of the assay medium in serologic test tubes. Incubation was carried out at 28–30° C with constant shaking (Kahn type shaker; 275–285 oscillations per minute through 1½" stroke) as in all subsequent experiments. The population per unit volume of the yeast-like

fungi was found at intervals of hours, using the leukocyte chamber of the hemacytometer, until the values obtained approached the asymptote. With *M. fulvum* and *S. brevicaulis* 200 conidia were examined at intervals and the numbers of germinating cells recorded.

Counts of the total population in aliquots from replicate cultures of the yeast-like fungi varied more than counts on the blastosporulating fraction of the population only. It is believed that this failure of counts of total population to "check" resulted from premature mechanical separation of some of the blastospores due to the shaking. As the blastospores of *C. tropicalis* are multiple in number, the premature separation of some of them does not prevent the recognition of actively growing cells. Thus, with this organism the number of blastosporulating fungi and not the total number of cells, was selected as the more satisfactory measure of growth. To arrive at an estimate of the variation to be expected in such cell counts of yeast-like fungi, the mean number of blastospores per unit volume was found in 25 groups of three cultures of *C. tropicalis* which had been prepared as replicates. The coefficient of variation was 6.0%.

Assay Technic. In obtaining dosage-response data and estimating M values, the number of germinating individuals in 200 was found for *M. fulvum* and *S. brevicaulis*, and the number of budding individuals of *Candida* or *Saccharomyces* per cubic millimeter, for several levels of each drug studied, using the incubation intervals and counting procedures described above. Triplicate cultures of each microorganism at each level of the test compounds provided satisfactory values.

As dozens of culture tubes were incubated simultaneously in each experimental "run," it was necessary to stabilize their contents on removal from the incubator during the interval required to count the spores in all of them. This was accomplished by refrigerating the cultures at 4° C. It could also be accomplished by adding a measured drop of formaldehyde to each of the culture tubes.

EVALUATION OF TECHNIC

Several experiments were undertaken for the purpose of evaluating the technic.

(a) To estimate the extent to which the M values for a known antifungal compound are reproducible, four dose-response curves were obtained for Bradosol(R) with *C. tropicalis,* using different inoculum densities, and solutions of the drug separately prepared. Incubation was carried out for different lengths of time from 14 to 18 hours. The range of M values obtained was 0.8 ppm, with a mean for the four experiments of 2.2 ppm.

(b) A comparison of the sensitivity of the counting technic with that of photoelectric turbidimetry in the detection of early differences in the growth response of *C. tropicalis* was made. The Coleman spectrophotometer was utilized, employing a wavelength of 400 millimicra. The results of this comparison are tabulated below.

Growth of *C. tropicalis*

Measured by Photoelectric Turbidimetry and by Direct Cell Counts

Incubation time (hours)	*% Transmission*	*Blastospores per cubic millimeter*
0	99.8	32
4	100.0	60
6	100.0	115
8	100.0	190
11	99.6	458

Differences in growth were discernible by the counting technic seven hours earlier than by turbidimetry.

(c) It was also necessary to test whether indications of activity for certain drugs, obtained by direct cell counting, were verifiable by other methods of estimating growth response, and whether drugs active for *C. tropicalis* were also active for other kinds of fungi under the same and other conditions.

(1) The M value for the steroid compound 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide was estimated with *C. tropicalis* to be in the vicinity of 5 ppm. Cortisone acetate and pregnenolone, on the other hand, were inactive at this level, but at the levels of about 10 ppm and above, they accelerated the growth of this fungus. These three steroid compounds and two other active compounds, undecylenic acid and 3,4-dichloro-ω-tri-

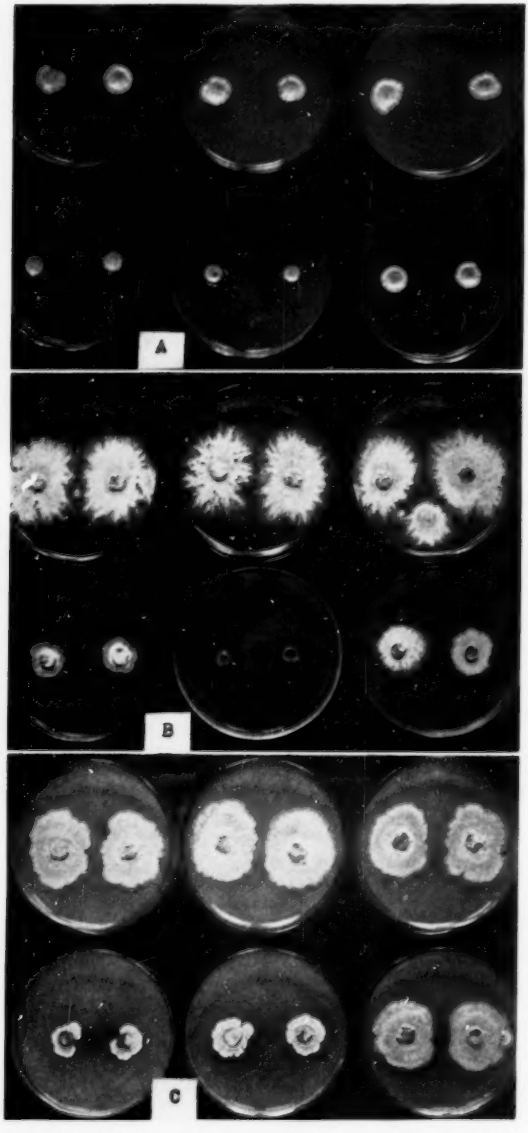

FIG. 1. Growth on Sabouraud's agar plates as a measure of drug activity. (A) *C. tropicalis*, (B) *M. fulvum*, (C) *S. brevicaulis*. From left to right the plates contained 100 ppm of each of the following: no drug, cortisone acetate, pregnenolone, 3,4-dichloro-ω-trichloromethyl acrylophenone, 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide, undecylenic acid.

chloromethyl acrylophenone, were incorporated separately in agar plates. The growth of *C. tropicalis*, *M. fulvum* and *S. brevicaulis* on these plates in the presence of 100 ppm of each compound is shown in FIG. 1. The general response of the microorganisms to the drugs was the same as by the counting technic with *C. tropicalis*. Growth on the agar plates appeared to be a less sensitive gauge of direction and extent of drug activity. Enhancement of the plate cultures by cortisone and pregnenolone could just be discerned.

(2) Two of the steroid compounds which exhibited activity as measured by cell counts with *C. tropicalis* and three commercial antimicrobial agents, were tried against *M. fulvum*, *S. cerevisiae* and *S. brevicaulis* as well, at levels 1 and 100 ppm. The values are given in blastospores per cubic millimeter for *C. tropicalis* and *S. cerevisiae*, and in germinating conidia per 200 for *M. fulvum* and *S. brevicaulis* in the table.

EFFECTS OF ANTIFUNGAL SUBSTANCES ON THE GROWTH OF FOUR FUNGI

Substance	*C. tropicalis*			*M. fulvum*			*S. brevicaulis*			*S. cerevisiae*		
	Parts per million of substances in cultures											
	0.0	1.0	100.0	0.0	1.0	100.0	0.0	1.0	100.0	0.0	1.0	100.0
Orthocide(R)	778	635	12	34	6	0	116	146	125	465	155	115
Triton A(R)	535	492	471	50	71	40	146	143	146	228	132	105
Vioform(R)	535	544	16	50	45	0	146	156	0	228	28	15
17-amino-Δ^4-androstene-3-one HCl	535	720	58	50	29	10	146	146	125	228	130	107
20-amino-3β-hydroxy-5-pregnene HCl	535	329	39	50	53	9	146	156	72	228	140	36

The pattern of response was similar in all four organisms; the non-pathogens were somewhat less sensitive as indicators.

EXPERIMENTAL RESULTS

Screening of Test Compounds. Measurement of the growth of the test organisms in the presence of 1 and 100 ppm of the various steroids and of the non-steroid compounds which were included for comparison, resulted in the values listed in TABLE I.

TABLE I

GROWTH OF CANDIDA TROPICALIS AND MICROSPORUM FULVUM WITH STEROID AND OTHER COMPOUNDS

Compounds (Grouped according to activity)	*C. tropicalis**			*M. fulvum***		
	Parts per million of compound in culture medium					
	0.0	1.0	100.0	0.0	1.0	100.0
(a) Stimulation						
Cortisone acetate	1192	1235	2215	50	96	100
Hydrocortisone acetate	710	1122	1030	72	72	76
Testosterone	1192	1320	1578	72	81	100
Testosterone-diethyl-aminoethyl carbonate	1075	1209	2198	72	75	80
(b) Equivocal						
Cholesterol	535	540	476	50	78	96
Pregnenolone	1192	1216	1772	72	72	69
17-amino-Δ^5-androstene-3-ol	1192	1170	1568	72	65	46
Testosterone undecylenate	1075	1166	1568	72	68	58
(c) Slight Inhibition						
α-estradiol	1192	1186	1082	72	70	68
Desoxycorticosterone glucoside	1075	951	562	72	72	66
Diethyl-2-hydroxyethyl-(3-keto-Δ^4-17-pregnadienyl) NH_4Br	1075	1232	518	72	71	42
Progesterone	1075	1047	998	76	70	64
(d) Moderate Inhibition						
Desoxycorticosterone	665	695	365	72	64	0
17-amino-Δ^5-androstene-3-one HCl	1070	1059	116	50	34	10
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr	1192	1140	188	72	55	48
20-amino-3β-hydroxy-5-pregnene HCl	1070	658	78	50	23	9
(e) Non-steroid Compounds						
Bradosol(R)	945	427	0	50	20	0
o-dichloroacetyloxine	1015	706	0	50	31	0
3,4-dichloro-ω-trichloromethyl acrylophenone	1015	698	0	50	29	0
Undecylenic acid	910	1170	610	52	48	0

* Values represent numbers of blastospores per cubic millimeter of culture.

** Values represent numbers of germinating macroconidia in two hundred.

Enhancement of fungus growth by antimetabolites frequently occurs at levels lower than those which are toxic (**41**). It may be this effect which is seen with the compounds of group (a) in TABLE I. The effect emerges more clearly with the steroids of group (b). The critical level for inhibition of the indicator organisms by the compounds of group (b) evidently lies in the re-

gion of 100 ppm. Acceleration of the growth of one of the indicators and inhibition of the other may be noted with these compounds. A number of steroids (Groups(c) and (d)) possessed some antifungal activity.

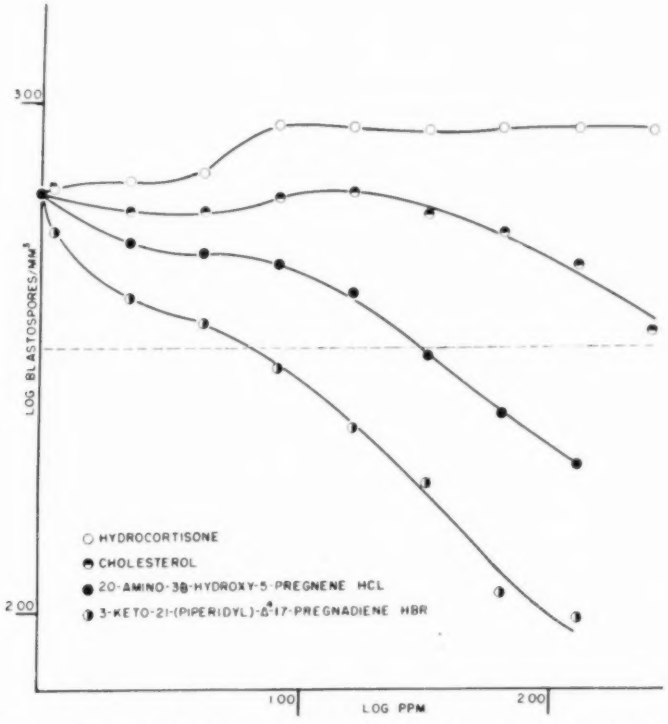

FIG. 2. *C. tropicalis.* Dosage-response curves for some active steroids. The broken line intersects the curves at the M values.

Comparison of M Values. Using *C. tropicalis* as indicator, dosage-response data were obtained in more detail for cholesterol, hydrocortisone acetate, testosterone-diethyl-aminoethyl carbonate, the steroid compounds of group (d) and the non-steroids of group (e) (TABLE I). The acceleration of growth in the presence of hydrocortisone acetate as indicated in TABLE I was confirmed (FIG. 2). A similar acceleration was seen with testosterone-diethyl-aminoethyl carbonate. Median effective dose estimates

for desoxycorticosterone, 17-amino-Δ^4-androstene-3-one monohydrochloride and cholesterol were found to be in the vicinity of or greater than 100 ppm. Only five compounds of the entire group possessed any notable activity against *C. tropicalis*. The M values obtained for these five substances were as follows:

Compound	*Median effective dose ppm*
Bradosol(R)	2
o-dichloracetyl oxine	1
3,4-dichloro-ω-trichloromethyl acrylophenone	3
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr	5
20-amino-3β-hydroxy-pregnene HCl	25

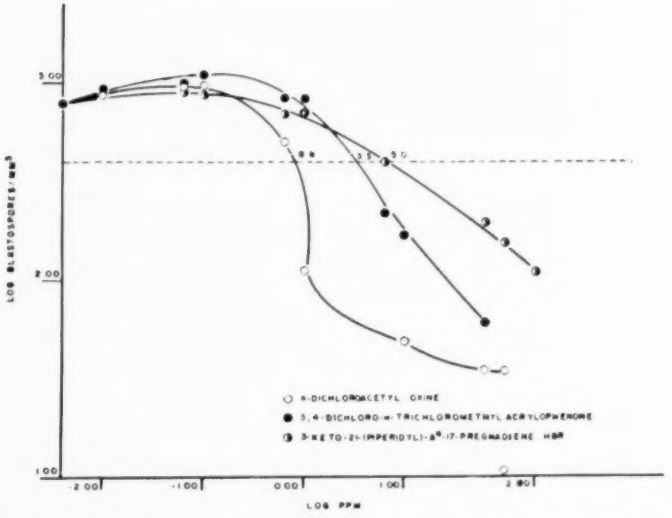

FIG. 3. Dosage response of *C. tropicalis* to three antifungal compounds. The broken line intersects the curves at the M values.

Effects of Mixtures of Antifungal Substances on the Growth of C. tropicalis. (a) Dosage-response data were obtained for *o*-dichloroacetyl oxine, 3,4-dichloro-ω-trichloromethyl acrylophenone, and the steroid, 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide, employing three different media. The media were the assay broth, and two portions of the same broth to which were

added sufficient Bradosol(R) to give concentrations of 0.1 and 10.0 ppm, respectively.

A comparison of the response curves in FIGS. 3 and 4 discloses that in the presence of a trace of Bradosol(R) the M values obtained for the other three compounds were somewhat lower than in basal medium only. Traces of this fungicide in the medium enhanced the activity of the other three drugs to a moderate degree.

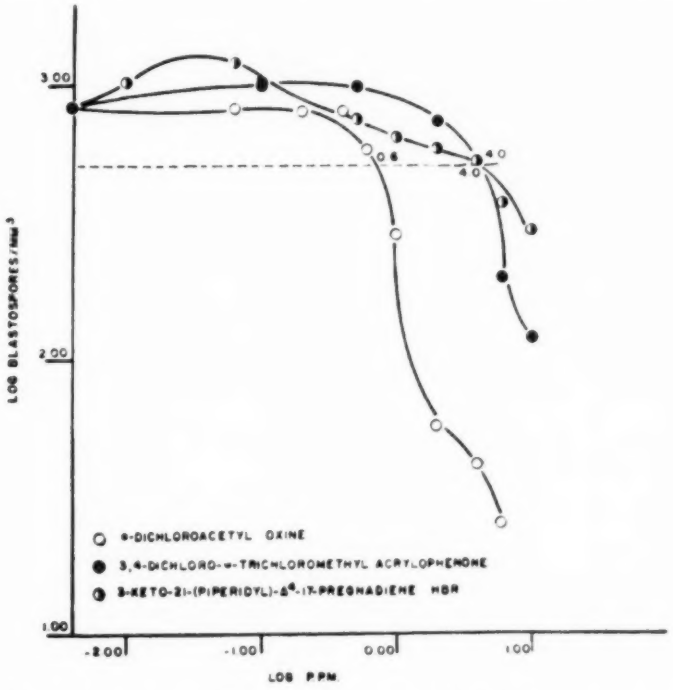

FIG. 4. Dosage-response of *C. tropicalis* to three antifungal compounds in medium containing 0.1 ppm Bradosol(R). The broken line intersects the curves at the M values.

In the presence of 10 ppm (approximately 5 M) of Bradosol(R), the M values for each of the other three drugs were considerably lower (FIG. 5) than in the basal medium. In addition, a curious effect was seen. There was now a choice of M values as follows:

Compound	Median effective dose (ppm) Lower dose	Higher dose
o-dichloroacetyl oxine	0.02	1.0
3,4-dichloro-ω-trichloromethyl acrylophenone	0.05	2.0
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr ..	0.09	1.0

The advantages seemingly offered by the low median effective doses for the three drugs in this experiment are illusory. Approximately five M of the Bradosol(R) compound were present in the assay broth. Mixture of any of the other three drugs in amounts up to one or two parts per million with 10 ppm of Bradosol(R) interfered with the fungicidal action of that compound.

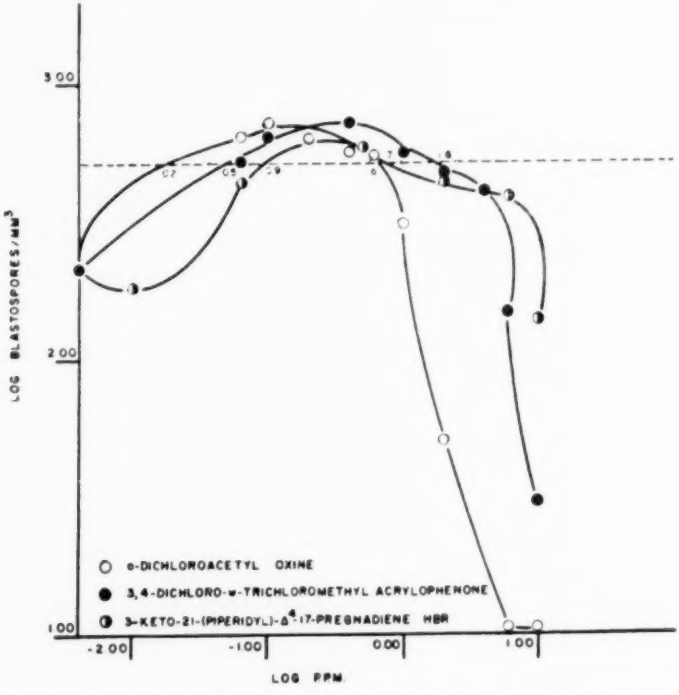

FIG. 5. Dosage-response of *C. tropicalis* to three antifungal compounds in medium containing 10 ppm Bradosol(R). The broken line intersects the curves at the M values.

(b) Dosage-response data for *C. tropicalis* with 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide were obtained in basal medium and in media containing, respectively, 0.1 and 10.0 ppm of 20-amino-3β-hydroxy-5-pregnene monohydrochloride (FIG. 6).

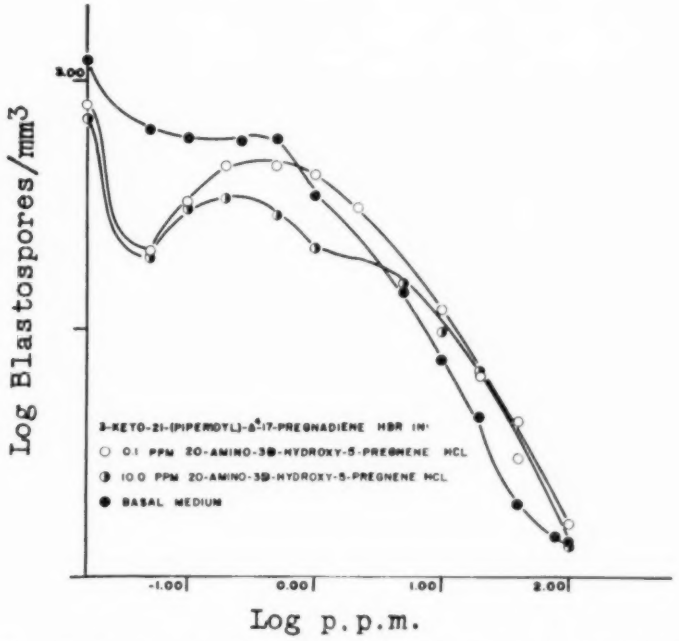

FIG. 6. *C. tropicalis*. Dosage-response to 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in basal medium and media containing 20-amino-3β-hydroxy-5-pregnene monohydrochloride.

Nothing appeared to be gained by admixture of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in amounts exceeding one or two parts per million with either 0.1 or 10.0 ppm of the other steroid. But mixture of fractional parts per million with either 0.1 or 10.0 ppm of 20-amino-3β-hydroxy-5-pregnene monohydrochloride resulted in antifungal activity greater than the activities of either of the two compounds alone at any of these levels.

(c) Dosage-response data for *C. tropicalis* with 20-amino-3β-hydroxy-5-pregnene monohydrochloride were similarly obtained, employing basal medium and media containing, respectively, 0.1 and 10.0 ppm of the other steroid (FIG. 7). In this experiment, the most desirable synergistic effect was seen with the combination,

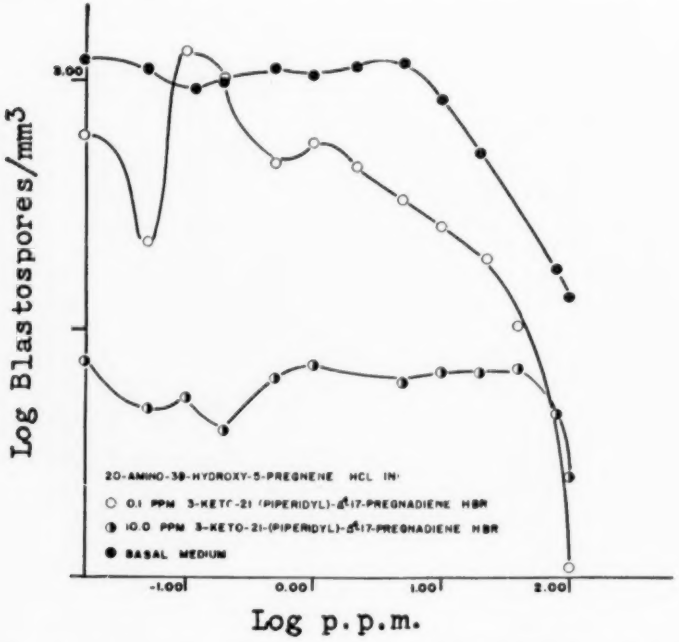

FIG. 7. *C. tropicalis*. Dosage-response to 20-amino-3β-hydroxy-5-pregnene monohydrochloride in basal medium and media containing 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide.

0.05 ppm of the first pregnene derivative with 0.1 ppm of the second. The antifungal activity of 20-amino-3β-hydroxy-5-pregnene monohydrochloride was enhanced to a considerable extent at almost all levels by the inclusion of 0.1 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in the medium.

In medium containing 10 ppm (approximately 2 M) of the second compound, on the other hand, little or nothing was gained by the addition of 20-amino-3β-hydroxy-5-pregnene monohydrochloride.

(d) To observe whether some of the drug combinations found to act in synergism or antagonism with *C. tropicalis* would affect the growth of another fungus similarly, Sabouraud's agar plates containing some of the combinations were prepared, and the growth on them of *M. fulvum* was observed (FIGS. 8, 9). These plates were inoculated with discs cut with a No. 3 cock-borer from 3-weeks-old colonies of *M. fulvum* on Sabouraud's agar.

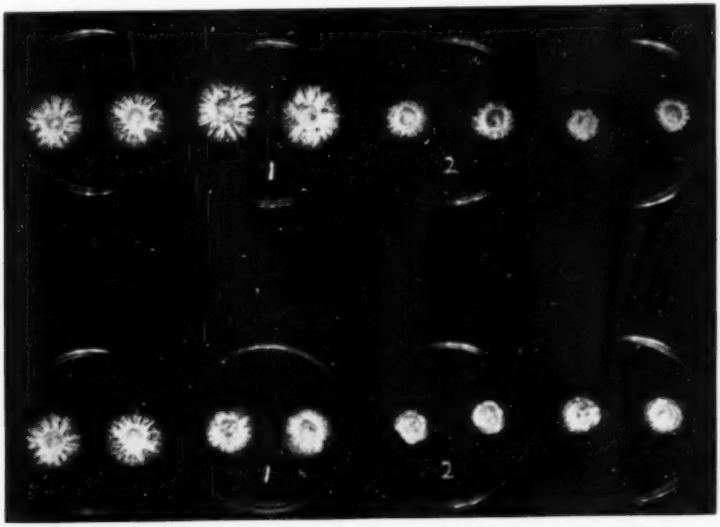

FIG. 8 (above). Drug synergism with *M. fulvum*. Left to right: control; 0.05 ppm 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide; 10 ppm 21-amino-3β-hydroxy-5-pregnene monohydrochloride; (1) plus (2).

FIG. 9. (below) Drug antagonism with *M. fulvum*. Left to right: control; 2 ppm Bradosol[®]; 5 ppm 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide; (1) plus (2).

The dermatophyte evidenced particular sensitivity to 20-amino-3β-hydroxy-5-pregnene monohydrochloride. A quantity less than half the M value for *C. tropicalis* inhibited radial growth considerably. Combination of 10.0 ppm of this steroid with 0.05 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide (a trace which by itself actually accelerated the growth of the fungus) affected the development of the organism more than either drug

alone (FIG. 8). With combinations of 2.0 ppm of Bradosol(R) with 5.0 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pragnadiene monohydrobromide, another effect was seen. The separate antifungal activities of the drugs were not additive. The combination produced an intermediate degree of inhibition (FIG. 9).

DISCUSSION

The aim of the laboratory assay of the fungicidal activity of chemical compounds is to distinguish and measure differences in potency and to reduce these differences to figures of performance which can be conveniently compared. Our trial of *Candida tropicalis* as a test organism has shown this pathogen to be sensitive and sufficiently manageable as an indicator to possess real value as a tool in the estimation and comparison of the potencies of a variety of compounds and mixtures.

Several steroids which were assayed in these experiments were found to stimulate the growth of the fungi at relatively low concentrations, while interfering with their development at higher levels. Wooley (**41**), Foster (**7**) and others have put forward explanations of this capacity of many antimetabolites to stimulate growth at sub-toxic levels. Some of the steroids, including cortisone and hydrocortisone, consistently accelerated the growth of the cultures in the concentrations employed. Probably this increase of growth bears no relation to the enhancement of fungus infections which has occasionally been noted in connection with cortisone therapy.

It would not be rewarding to undertake a full discussion of the many mechanisms whch have been proposed to explain the occasional enhancement of infection with cortisone. It should be pointed out, however, that delay of the inflammatory reaction (**21, 22, 23**), interference with the normal processes of healing (**27**), and interference in general with the function of the reticuloendothelial and lymphatic systems (**34, 39**), have been among the explanations offered. Most authorities agree that cortisone acts in some way to increase host susceptibility to infectious microorganisms, but has no direct influence on the agents of disease *in vivo*.

If the dosage-response curves obtained for all of the steroids were arrayed as in FIG. 2, it was seen that cholesterol itself inhibited the test organisms to some extent, and that the nitrogen-containing steroids possessed the greatest activity. Further correlation to the differing activities with the different structures of the steroids would be difficult. Probably the cyclopentanophenanthrene nucleus itself contributes to the antifungal properties of some of these compounds. It is known that few fungi are able to oxidize the steroid molecule (**36**, **37**), and that some of the many steroid substances synthesized by fungi themselves represent waste products (**7**) capable of limiting the growth of the organisms which produce them. It is thus not surprising that some mammalian steroid hormones and related substances, analogous in structure to natural mycosterols also affect the growth of fungi.

Only a few of the steroids possessed any considerable antifungal potency. These were desoxycorticosterone, 17-amino-Δ^4-androstene-3-one-monohydrochloride, 20 amino-3β-hydroxy-5-pregnene monohydrochloride and 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide. The slopes of the dosage response curves obtained with these compounds were gradual, unlike the steep "drops" seen with protoplasmic poisons and other highly toxic fungicides. Such slopes have been associated with antifungal agents possessing the differential toxicity for host organism and fungus parasite which characterizes many useful antibiotics. Further study of antifungal steroids, including animal experimentation, is, of course, essential before conclusions concerning their practical usefulness can be drawn. It is of interest that Kull *et al.* (**42**) have also recently found that these and other amino steroids possess antimicrobial activity.

Some synergistic effects of interest were seen when, instead of single compounds, mixtures of some of the drugs were assayed. A sub-toxic trace of Bradosol(R) induced lower M values when combined with certain other drugs. Admixture of Bradosol(R) with other drugs in a quantity exceeding its own M value resulted, on the other hand, in reduced antifungal activity. This appears to be an instance illustrating the common observation that synergism may occur at some relative proportions and antagonism

at other proportions with the same compounds. There was also synergistic inhibition of the indicator microorganisms by mixtures of 3-keto-21-(piperidyl)-Δ^5-17-pregnadiene monohydrobromide and 20 amino-3β-hydroxy-5-pregnene monohydrochloride (FIGS. 6, 7). Of particular interest were the antifungal effects of mixtures containing traces of these steroids so slight as to be inactive separately. It seems possible that they operate as mutual activators.

SUMMARY

The antifungal activities of a number of steroids and other compounds were compared by a spore-counting method, employing *Candida tropicalis* as the principal test organism. This organism proved to be of particular value as an indicator in these tests.

While most of the steroid compounds possessed some activity, only a few exhibited any notable property as fungicides. With the exception of desoxycorticosterone, the active steroids were nitrogen-containing derivatives of sex hormones.

A few steroid hormones, including cortisone, accelerated the growth of the fungi. Probably this acceleration is not related to the enhancement of fungus infections which has sometimes been noted in connection with cortisone therapy for the relief of other conditions.

Certain mixtures of the steroids and other compounds inhibited the growth of the fungi synergistically. In other mixtures, the antifungal potencies of the components were antagonistic.

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DIFFERENTIATION OF SPECIES OF TAPHRINA IN CULTURE. UTILIZATION OF NITROGEN COMPOUNDS

A. J. Mix

A number of species and of host-forms of *Taphrina* have been brought into pure culture over a period of several years. Since various species and various isolates show only minor differences in colony-characters, and many of them are much alike in morphology of cells formed in culture, other methods of differentiation need to be sought.

In their characterization of genera and species of yeasts, Stelling-Dekker (**5**), Lodder (**1**) and other workers have included the ability of the fungi to utilize certain compounds of nitrogen. Species of *Taphrina* in culture behave as yeasts. An unknown species of *Taphrina* in culture would in fact have to be identified as a species of *Torulopsis* as defined by Lodder (l.c.) or by Lodder and Kreger-van Rij (**2**). Nothing yet published gives any suggestion as to a means of differentiating between the genera *Taphrina* and *Torulopsis* as they appear in culture.

The purpose of the work reported here was to learn whether utilization of various compounds of nitrogen could be used as a basis for differentiation between species of *Taphrina* and between host-forms within one species. It was also hoped to discover whether any significant differences existed between species of *Taphrina* and certain species of non-sporulating yeasts.[1]

[1] A preliminary report of this experimentation was read at the Seventh International Botanical Congress at Stockholm in 1950 and a summary of results is being published in the Proceedings of the Congress. Since 1950, with repetition of a number of experiments some compounds believed to be unavailable to certain fungi have been found usable. The summary mentioned above is therefore incorrect in certain details, but statements made regarding agreement or disagreement in behavior between different species and different host forms do not require correction.

The choice of these yeasts was prompted by the comparative studies made by Roberts (4) with *Taphrina deformans* and *Torulopsis pulcherrima*. The intent was to include representative species of the genus *Torulopsis*. However *Torulopsis pulcherrima* was transferred by Lodder (l.c.) to the genus *Candida*. Of the twelve other fungi chosen six have been recently assigned by Lodder and Kreger van-Rij (2) to the genus *Cryptococcus*, and two to *Candida*. The remaining four are species of *Torulopsis* though the culture of *T. rotundata* used, since it was obtained from Centraalbureau voor Schimmelcultures, may have been the culture studied by Lodder and Kreger-van Rij (l.c.) and found by them to be a strain of *Cryptococcus diffluens* (Zach) Lodder and Kreger-van Rij.

Cultures used: In the following list of cultures those designated by number were used in all the experiments. Additional isolates, usually made at the same time, were used in a number of cases where experiments were repeated. These additional isolates are designated by letter as a, b, etc. in parentheses following the culture number. Original isolations were by the author, unless otherwise designated.

Culture No. 1. (a) *T. americana* Mix. *Betula fontinalis* Sarg.
2. *T. betulina* Rostr. *Betula intermedia* Thomas.
3. (a) *T. betulina*. *B. intermedia*. Formerly *T. Lapponica* Juel.
4. (a) *T. betulina*. *B. pendula* Roth. Formerly *T. turgida* (Sadeb.) Giesenhag.
5. *T. caerulescens* (Mont. and Desm.) Tul. *Quercus alba* L.
6. *T. caerulescens*. *Q. bushii* Sarg.
7. *T. caerulescens*. *Q. coccinea* Muench.
8. *T. caerulescens*. *Q. ilicifolia* Wangh.
9. *T. caerulescens*. *Q. kelloggii* Newb. Isolated by H. N. Hansen.
10. *T. caerulescens*. *Q. laurifolia* Michx.
11. *T. caerulescens*. *Q. macrocarpa* Michx.
12. *T. caerulescens*. *Q. marilandica* Muench.
13. *T. caerulescens*. *Q. maxima* Ashe.
14. *T. caerulescens*. *Q. maxima*.
15. *T. caerulescens*. *Q. nigra*. L.
16. *T. caerulescens*. *Q. palustris* Moench.
17. *T. caerulescens*. *Q. prinoides* L.
18. *T. caerulescens*. *Q. velutina* Lam.
19. *T. caerulescens*. *Q. velutina*.

20. *T. caerulescens.* *Q. virginiana* Mill.
21. *T. carnea* Johans. *Betula intermedia* Thomas.
22. *T. cerasi* (Fkl.) Sadeb. Obtained in 1935 from Centraalbureau voor Schimmelcultures. Originally isolated by M. Wieben. Probably from *Prunus avium* L. but host not definitely known.
23. *T. cerasi.* *P. avium.*
24. (a) *T. cerasi.* *P. avium.*
25. (a) *T. cerasi.* *P. pennsylvanica* L.
26. *T. cerasi.* *P. pennsylvanica.*
27. (a, b) *T. communis* (Sadeb.) Giesenhag. Fruits of *Prunus americana* Marsh.
28. (a) *T. communis.* Fruits *P. americana.*
29. (a) *T. communis.* Twigs *P. americana.* Formerly *T. decipiens* (Atk.) Giesenhag.
30. *T. communis.* Twigs *P. angustifolia* Marsh. Formerly *T. mirabilis* (Atk.) Giesenhag.
31. *T. communis.* Fruits *P. angustifolia.* Formerly *T. mirabilis* (Atk.) Giesenhag., var. *tortilis* Atk.
32. (a) *T. communis.* Fruits *P. angustifolia.* Formerly *T. mirabilis* var. *tortilis.*
33. (a) *T. communis.* *P. lanata* (Sudw.) Mack. and Bush.
34. *T. communis.* *P. maritima* Marsh.
35. (a) *T. communis.* *P. nigra* Ait.
36. (a) *T. communis.* *Prunus* sp., cultivated plum. Kansas.
37. *T. communis.* *Prunus* sp., cultivated plum. Virginia.
38. *T. confusa* (Atk.) Giesenhag. *P. virginiana* L.
39. *T. confusa.* *P. virginiana* L. var. *demissa* Torr.
40. *T. dearnessii* Jenkins. *Acer rubrum* L.
41. *T. deformans* (Berk.) Tul. *Prunus persica* L.
42. (a) *T. deformans.* *P. communis* L.
43. *T. epiphylla* Sadeb. *Alnus incana* Moench.
44. (a) *T. epiphylla.* *A. incana.* Formerly *T. kiebahni* Wieben.
45. (a) *T. farlowii* Sadeb. *Prunus serotina* Thrh.
46. (a) *T. farlowii.* *P. serotina.*
47. (a) *T. flavorubra* Ray. *P. pumila* L. var. *susquehanae* Jaeg.
48. *T. flavorubra.* *P. besseyi* Bailey.
49. *T. johansonii* Sadeb. *Populus tremuloides* Michx.
50. (a) *T. johansonii.* *P. tremuloides.*
51. *T. letifera* (Pk.) Sacc. *Acer spicatum* Lam.
52. *T. nana* Johans. *Betula nana* L.
53. *T. polystichi* Mix. *Polystichum acrostichoides* (Michx.) Schott.
54. *T. polystichi.* *P. acrostichoides.* Received in 1939 from W. W. Ray, and isolated by him.
55. *T. populina* Fr. *Populus nigra* L. Isolated by M. Wieben. Received in 1935 from Centraalbureau voor Schimmelcultures.

56. (a) *T. populina*. *P. nigra*.
57. (a) *T. populi-salicis* Mix. *P. fremontii* S. Wats.
58. (a) *T. populi-salicis*. *P. trichocarpa* Torr. and Gray.
59. (a) *Taphrina potentillae* (Farl.) Johans. *Potentilla arguta* Pursh.
60. *T. potentillae*. *P. canadensis* L. from E. M. Martin, and isolated by her. Received in 1931.
61. (a) *T. potentillae*. *P. canadensis*.
62. *T. pruni-subcordatae* (Zeller) Mix. *Prunus subcordata* Benth.
63. *T. purpurascens* Robins. *Rhus copallina* L. Isolated by C. L. Kramer.
64. (a, b) *T. robinsoniana* Giesenhag. *Alnus rugosa* (Du Roi) Spreng.
65. *T. robinsoniana*. *A. rugosa*.
66. *T. robinsoniana*. Catkin *A. serrulata* (Ait.) Willd.
67. (a) *T. robinsoniana*. Catkin *A. serrulata*. Formerly *T. rugosa* Ray.
68. *T. robinsoniana*. Catkin *A. serrulata*.
69. *T. robinsoniana*. Leaf *A. serrulata*.
70. (a) *T. sacchari* Jenkins. *Acer saccharum* Marsh.
71. *T. sacchari*. *A. saccharum*.
72. (a) *T. sadebeckii* Johans. *Alnus glutinosa* Gaertn.
73. (a) *T. sadebeckii*. *A. serrulata* (Ait.) Willd.
74. *T. tosquinetii* (Westend.) Tul. *A. glutinosa*. Isolated by M. Wieben. Received in 1935 from Centraalbureau voor Schimmelcultures.
75. (a) *T. tosquinetti*. *A. glutinosa*.
76. *T. ulmi* (Fkl.) Johans. *Ulmus alata* Michx.
77. *T. ulmi*. *U. rubra* Muhl.
78. *T. ulmi*. *U. rubra*.
79. (a) *T. virginica* Sadeb. *Ostrya virginiana* (Mill.) K. Koch.
80. *Cryptococcus albidus* (Saito) Skinner. Obtained from Centraalbureau voor Schimmelcultures.
81. *Torulopsis colliculosa* (Hartm.) Sacc. From C.B.S.
82. *T. dattila* (Kluyver) Lodder. From C.B.S.
83. *Cryptococcus diffluens* (Zach) Lodder and Kreger-van Rij. From C.B.S.
84. *C. laurentii* (Kuff.) Skinner var. *flavescens* (Saito) Lodder and Kreger-van Rij. From C.B.S.
85. *Candida pseudotropicalis* (Cast.) Basgal var. *lactosa* (Harrison) Diddens and Lodder. Obtained as *Torulopsis kefyr* (Beij.) Lodder from U.S.D.A. Northern Regional Res. Lab., Peoria, Ill. Their culture y-196.
86. *Cryptococcus laurentii* (Kuff.) Skinner. From C.B.S.
87. *C. luteolus* (Saito) Skinner. From U.S.D.A.N.R.R.L.
88. *C. neoformans* (Sanfelice) Vuill. From C.B.S.

89. *Candida pulcherrima* (Lindner) Windisch. Obtained from Catherine A. Roberts. Isolated by E. M. Mrak.
90. *C. pulcherrima.* From California grapes.
91. *Torulopsis rotundata* Redaelli. From C.B.S.
92. *T. sphaerica* (Hammer and Cordes) Lodder. From C.B.S.
93. *Candida utilis* (Henneberg) Lodder and Kreger-van Rij. From C.B.S.

In the experiments all cultures were made in duplicate, in 15 × 125 mm tubes, each tube containing 4.5 ml of the following medium: KH_2PO_4 2.75 gm, $MgSO_4 \cdot 7H_2O$ 1.25 gm, dextrose 10.0 gm, distilled water 1000 ml. To this was added the particular nitrogen-source.

Inorganic nitrogen compounds were used in amounts furnishing the equivalent in nitrogen to 2.00 gm KNO_3 per liter. Peptone was used in a concentration of 2.00 gm per liter. Following experiments in which KNO_3 and asparagine were used at half strength with no reduction or with an increase in growth, the organic compounds: urea, asparagine, amino acids, were used at half strength (KNO_3-equivalent 1 gm per liter). This rendered the growth-values obtained with inorganic and organic compounds not comparable. (See later discussion of amount of growth.)

To a second set of cultures in each experiment thiamine hydrochloride was added in a concentration of one part per million. Other growth-promoting substances and trace-elements were omitted from the solutions since the object was to differentiate between organisms rather than to study the nutrition of any of them. Also the fact that many of the nitrogen-bearing compounds used contained carbon was not considered important in view of the purpose of the experiments.

Experiments with the following compounds were repeated: ammonium chloride, potassium nitraté, l-arginine monohydrochloride, asparagine, dl-aspartic acid, l-glutamic acid, dl-histidine HCl. Partial repetitions of other experiments were made in all cases (except dl-threonine) where compounds were refused by particular organisms. In these partial repetitions additional isolates of each fungus were employed if any were available.

The initial hydrogen-ion concentration of the medium was in most cases in the neighborhood of pH 4.5, this having been previously found favorable for the growth of *Taphrina deformans* and *T. communis* in certain media. In a few cases the media as made up had concentrations as high as pH 4.8 and in one case (dl-histidine HCl) pH 5.60. These media were not adjusted. Certain highly acid media were adjusted by the addition of suitable amounts of K_2HPO_4 or by the substitution of that compound for KH_2PO_4. Adjustment was made to a concentration near pH 4.5.

Final hydrogen-ion concentration was recorded in each case. Usually the degree of shift in a given direction could be taken as a measurement of the amount of growth and served to substantiate the observations of growth made in other ways. Cultures were kept at room temperature and results were taken at the end of 21 days. Amount of growth obtained was estimated by visual observation of the degree of turbidity of the solution caused by the presence of the suspended cells. These observations were expressed in symbols as follows: O (no visible growth) or tr (such slight growth as might be due to impurities in the medium); + (definite but slight growth); ++ (good growth); +++ (very good growth, comparable to that obtained with potato dextrose broth).

Amount of turbidity was also measured by means of an electrophotometer. It was not found possible to correlate the readings obtained on this instrument with cell-counts made with a haemocytometer. All organisms studied resemble yeasts in that they form in culture series of attached cells or buds. These "colonies" render accurate cell counts impossible. Therefore, since the electrophotometer readings could have only relative value, they were used only to substantiate visual observation in deciding which of the above-named symbols to employ.

Amount of growth did not actually become of much significance in these experiments and will not be reported in detail. Frequently different isolates of the same fungus made different amounts of growth in the same medium and in certain repetitions of experiments the same isolate made greater or less growth on the second trial.

Some of the growth-differences obtained on repeated trials with the same fungi are shown in TABLE I.

Detailed results of the experiments showing amounts of growth obtained in each case will not be presented. The report of a single experiment will indicate the kinds of results obtained, and will also

TABLE I

RESULTS OBTAINED IN REPEATED TRIALS OF DIFFERENT COMPOUNDS WITH DIFFERENT SPECIES OF TAPHRINA

Culture	Nitrogen compound	Trial no.	Growth without thiamine	Growth with thiamine
No. 35, *T. communis*	Potassium nitrate	1	tr	+ + +
		2	+ +	+ +
	Sodium nitrate	1	tr	tr
		2	+	+ +
		3	+	+ + +
	dl-alpha alanine	1	+ +	+ +
		2	+ + +	+ + +
	l-glutamic acid	1	tr	+ + +
		2	+ + +	+ + +
No. 27, *T. communis*	Potassium nitrate	1	+	+ + +
		2	+ +	+ + +
		3	+	+ + +
	l-arginine monohydrochloride	1	+	+
		2	+	+ +
No. 37, *T. communis*	Potassium nitrate	1	+	+ + +
		2	+	+
	dl-aspartic acid	1	+	+
		2	+	+ +
No. 12, *T. caerulescens*	l-arginine monohydrochloride	1	+	+
		2	+	+ +
No. 25, *T. cerasi*	Ammonium tartrate	1	+ +	+ +
		2	+	+ + +

present a list of the nitrogen compounds used. This experiment is reported in TABLE II.

Aside from amount of growth, actual utilization or refusal of various nitrogen compounds seemed, in these experiments, to be characteristic of the different species and host forms. Consideration should be given, however, to the question whether different isolates of the same species behave alike. It would seem possible that biochemical variants might exist among different isolates from the same host, even among those made at the same time. Further a number of these fungi had been maintained in culture for several years. As Roberts (**4**) has observed sectoring, in-

TABLE II

GROWTH MADE BY CULTURE NO. 10, TAPHRINA CAERULESCENS FROM QUERCUS LAURIFOLIA, WITH VARIOUS SOURCES OF NITROGEN

Source of nitrogen	Initial pH	Without thiamine		With thiamine	
		Growth	Final pH	Growth	Final pH
Ammonium acetate	4.86	0	—	0	—
Ammonium citrate	4.70	++	4.47	+++	4.08
Ammonium chloride	4.50	+++	3.43	+++	2.50
Ammonium nitrate	4.50	+++	2.90	+++	3.22
Ammonium oxalate	5.15	++	4.40	++	4.78
Ammonium phosphate	4.38	+	3.66	++	2.94
Ammonium sulfate	4.50	0	+	0	—
Ammonium tartrate	5.10	+	4.32	+++	3.87
Calcium nitrate	4.30	tr	—	tr	—
Magnesium nitrate	4.45	tr	—	+	4.30
Potassium nitrate	4.50	tr	4.60	++	6.10
Sodium nitrate	4.40	++	3.40	++	2.90
Peptone	4.80	+	3.72	++	4.34
Urea	4.50	++		++	5.27
dl-alpha alanine	4.80	++	4.00	+	4.70
Beta alanine	4.80	0	—	0	—
l-arginine monohydrochloride	4.40	+	3.28	+++	2.62
dl-asparagine	4.52	+	3.80	+++	4.74
dl-aspartic acid	4.30	++	3.32	+++	3.40
l-cysteine hydrochloride	4.32	0	—	0	—
l-glutamic acid	4.50	+	3.30	+++	3.12
Glycine	4.60	tr	—	0	—
dl-histidine HCl	5.60	0	—	0	—
Hydroxy-l-proline	4.58	0	—	0	—
dl-isoleucine	4.60	0	—	0	—
dl-leucine	4.60	0	—	tr	—
dl-methionine	4.66	+	4.38	+	4.32
dl-beta phenylalanine	4.60	tr	—	tr	
l-proline	4.56	+	4.60	+	4.65
dl-serine	5.30	+	4.50	+	4.62
dl-threonine	4.62	0	—	0	—
dl-valine	4.50	+	4.52	+	4.40
Potato dextrose broth		++			

volving color change, in colonies of *Taphrina deformans*, it would seem not impossible that biochemical variants might arise in cultures of this and other species.

As will appear from examination of the list of cultures given above, of the 79 species or host-forms of *Taphrina used* in all the experiments 12 are represented by two different isolates. These are: *Taphrina caerulescens* from *Quercus maxima*, *T. caerulescens* from *Q. velutina*, *T. epiphylla*, *T. farlowii*, *T. johansonii*, *T. polystichi*, *T. populina*, *T. potentillae* from *Potentilla canadensis*,

T. robinsoniana from *Alnus rugosa*, *T. sacchari*, *T. tosquinetii*, *T. ulmi* from *Ulmus rubra*. The following were represented by three isolates each: *T. cerasi*, *T. communis* from *Prunus americana*, *T. communis* from *P. angustifolia*, *T. robinsoniana* from *Alnus serrulata*. In addition, as indicated by the letters following the culture number, 26 forms were represented by additional isolates, used whenever individual experiments were repeated. Two isolates of *Candida pulcherrima* were used throughout the experiments.

For the most part there was close agreement in the performance of different isolates of the same fungi, though sometimes one isolate made more growth than another with a particular compound. Exceptions were as follows: 1. With ammonium tartrate two cultures of *Taphrina robinsoniana* (No. 64 and No. 65) made slight (+) and moderate (++) growth respectively, while other cultures of this fungus (Nos. 66–69) made no growth (O) or a trace of growth (tr). 2. With dl-methionine, *Taphrina communis*, cultures No. 28 and No. 29, made only a trace of growth (tr) while all other isolates (No. 27, Nos. 30–37) made slight (+) but definite growth. 3. *T. communis*, culture No. 27, made slight growth with dl-leucine but a second isolate (No. 27a) of this fungus and all other isolates of *T. communis* (Nos. 28–37) refused this compound.

A special experiment was run to test the behavior of different isolates. The following nitrogen compounds were employed: ammonium chloride, ammonium phosphate and glycine. The fungi were: *Taphrina communis*, five isolates; *T. deformans*, five isolates; *T. robinsoniana* from *Alnus rugosa*, five isolates, the same fungus from *A. serrulata*, five isolates. Very close agreement in behavior was found between the different isolates. *Taphrina communis* made good use of ammonium chloride and glycine but slight use of ammonium phosphate; *T. deformans* used ammonium chloride and glycine slightly, and refused ammonium phosphate; *T. robinsoniana* from *Alnus rugosa* and from *A. serrulata* agreed in making good use of ammonium chloride and glycine, and in refusing ammonium phosphate.

All species and host-forms of *Taphrina* included in the experiments refused ammonium acetate and all of the non-sporulating yeasts but *Torulopsis dattila* used that compound. (This experiment was not repeated.) All of the fungi made good use of urea and asparagine, and all but one grew well with peptone. Therefore in reporting the behavior of the fungi in detail ammonium acetate, urea, peptone and asparagine will not be listed.

The following is a summary of the behavior of the fungi tested:

T. americana, culture No. 1, used: all ammonium compounds but ammonium chloride; calcium, magnesium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenyl alanine, l-proline. Refused peptone.

T. betulina, culture No. 2, used: all ammonium compounds; magnesium, potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-isoleucine (slightly), dl-leucine, dl-methionine, dl-beta phenylalanine (slightly), l-proline (slightly), dl-serine, dl-valine.

T. betulina ("*T. lapponica*"), culture No. 3, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. betulina ("*T. turgida*"), culture No. 4, used: ammonium chloride, citrate, nitrate, oxalate; all nitrates; l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. caerulescens from *Quercus alba*, culture No. 5, used: all ammonium compounds but ammonium tartrate; calcium nitrate (slightly), potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, glycine (slightly), l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, l-proline, dl-valine.

T. caerulescens from *Q. bushii*, culture No. 6, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine

HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. coccinea*, culture No. 7, used: all ammonium compounds; all nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. ilicifolia*, culture No. 8, used: all ammonium compounds; calcium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-threonine, dl-valine.

T. caerulescens from *Q. kelloggii*, culture No. 9, used: all ammonium compounds; magnesium, potassium and sodium nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. laurifolia*, culture No. 10, used: all ammonium compounds but ammonium sulfate; magnesium nitrate (slightly), potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-methionine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. macrocarpa*, culture No. 11, used: ammonium citrate, nitrate, oxalate; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl.

T. caerulescens from *Q. marilandica*, culture No. 12, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. maxima*, cultures No. 13 and 14, used: all ammonium compounds; all nitrates; dl-alpha alanine (slightly), l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. nigra,* culture No. 15, used: all ammonium compounds; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. palustris,* culture No. 16, used: all ammonium compounds; magnesium and potassium nitrates; sodium nitrate (slightly), l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. prinoides,* culture No. 17, used: ammonium citrate, nitrate, oxalate; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl, dl-beta phenylalanine, dl-serine, dl-valine.

T. caerulescens from *Q. velutina,* cultures No. 18 and No. 19, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride (slightly), l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. virginiana,* culture No. 20, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. carnea, culture No. 21, used: all ammonium compounds but ammonium phosphate; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. cerasi, cultures No. 22, No. 23, and No. 24, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. cerasi from *Prunus pennsylvanica,* cultures No. 25 and No. 26, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-

glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. communis from fruits of *Prunus americana*, cultures No. 27 and No. 28, and from twigs of *P. americana*, culture No. 29, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-beta phenylalanine, l-proline, dl-serine, dl-valine. Culture No. 27 made slight use of dl-leucine and dl-methionine.

T. communis from twigs of *P. angustifolia* ("*T. mirabilis*"), culture No. 30, and from fruits of *P. angustifolia* ("*T. mirabilis* var. *tortilis*"), cultures No. 31 and No. 32, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine (slightly), dl-methionine, dl-beta phenylalanine, dl-serine, dl-valine.

T. communis from *P. lanata*, culture No. 33, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenylalanine (slightly), l-proline, dl-serine, dl-valine.

T. communis from *P. maritima*, culture No. 34, used the same series of compounds as the preceding and, in addition, hydroxy-l-proline.

T. communis from *P. nigra*, culture No. 35, used the same series of compounds as culture No. 33.

T. communis from *Prunus* sp., cultures No. 36 and No. 37, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride (slightly), l-glutamic acid, glycine, dl-histidine HCl, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, dl-serine, dl-valine.

T. confusa from *P. virginiana*, culture No. 38, used: all ammonium compounds; all nitrates: dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-beta phenylalanine (slightly), dl-serine, dl-valine.

T. confusa from *P. dimissa*, culture No. 39, used: all ammonium compounds; calcium nitrate (slightly), magnesium, and potassium nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. dearnessii, culture No. 40, used all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. deformans from *Prunus persica*, culture No. 41, and from *P. communis*, culture No. 42, used: all ammonium compounds but ammonium phosphate; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl (slightly), dl-methionine, dl-valine.

T. epiphylla, cultures No. 43 and No. 44 (*"T. klebahni"*), used ammonium chloride, citrate, nitrate, oxalate; magnesium, potassium, and sodium nitrates; l-glutamic acid (slightly), dl-histidine HCl, dl-leucine, dl-valine.

T. farlowii, cultures No. 45 and No. 46, used ammonium chloride, citrate, nitrate, oxalate, sulfate (slightly); all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. flavorubra from *Prunus pumila* var. *susquehanae*, culture No. 47, used all ammonium compounds but ammonium phosphate; calcium, potassium, and sodium nitrates; beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. flavorubra from *P. besseyi*, culture No. 48, agreed with the preceding except for the use of magnesium nitrate and very slight use of beta alanine.

T. johansonii, cultures No. 49 and No. 50, used; all ammonium compounds but ammonium phosphate; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid (slightly), l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. letifera, culture No. 51, used: ammonium chloride, citrate, nitrate, oxalate, phosphate (slightly); magnesium, potassium, and sodium nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-beta phenylalanine (slightly), dl-serine, dl-valine.

T. nana, culture No. 52, used: ammonium chloride (slightly), citrate, oxalate; magnesium, potassium, and sodium nitrates; l arginine monohydrochloride, dl-aspartic acid (slightly), dl-histidine HCl, dl-leucine (slightly), dl-methionine (slightly), dl-valine. This culture made only slight use of asparagine.

T. polystichi, cultures No. 53 and No. 54, used: ammonium chloride, citrate, nitrate, oxalate, sulfate; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride (slightly), dl-aspartic acid (slightly), l-glutamic acid, dl-histidine HCl, dl-valine (slightly).

T. populina, cultures No. 55 and No. 56, used: ammonium chloride, citrate, nitrate, oxalate, phosphate; magnesium, potassium, and sodium nitrates; dl-aspartic acid, dl-methionine (slightly), l-proline, dl-valine.

T. populi-salicis from *Populus fremontii*, culture No. 57, used: all ammonium compounds; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, l-proline, dl-valine.

T. populi-salicis from *P. trichocarpa*, culture No. 58, used: all ammonium compounds; all nitrates; dl-alpha alanine (slightly), l-arginine monohydrochloride (slightly), l-glutamic acid, dl-histidine HCl (slightly), l-proline, dl-valine.

T. potentillae from *Potentilla arguta*, culture No. 59, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-isoleucine (slightly), dl-leucine, l-proline (slightly), dl-serine, dl-valine.

T. potentillae from *P. canadensis*, cultures No. 60 and No. 61, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine (slightly), dl-methionine, l-proline (slightly), dl-serine, dl-valine.

T. pruni-subcordatae, culture No. 62, used: all ammonium compounds; calcium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-histidine HCl.

T. purpurascens, culture No. 63, used all compounds but calcium, magnesium, and potassium nitrates.

T. robinsoniana from *Alnus rugosa*, cultures No. 64 and No. 65, used: all ammonium compounds (ammonium phosphate and tartrate slightly); all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. robinsoniana from *A. serrulata*, cultures No. 66, No. 67, No. 68, and No. 69, used the same compounds as the preceding.

T. sacchari, cultures No. 70 and No. 71, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. sadebeckii, cultures No. 72 and No. 73, used: ammonium chloride, citrate, nitrate, oxalate, phosphate, sulfate (slightly); magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine (slightly), l-proline, dl-valine.

T. tosquinetii, cultures No. 74 and No. 75, used: all ammonium compounds; potassium nitrate; l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, dl-leucine (slightly), dl-serine (slightly), dl-valine.

T. ulmi from *Ulmus alata*, culture No. 76, used: ammonium chloride, citrate, nitrate, oxalate, tartrate; all nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-beta phenylalanine, dl-serine, dl-valine.

T. ulmi from *U. rubra*, cultures No. 77 and 78, used: ammonium chloride, citrate, nitrate (slightly), oxalate, sulfate (slightly), tartrate; magnesium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-beta phenylalanine, l-proline, dl-valine.

T. virginica, culture No. 79, used all compounds but l-cysteine hydrochloride, dl-isoleucine, dl-leucine, dl-threonine.

Crytococcus albida, culture No. 80, used all compounds.

Torulopsis colliculosa, culture No. 81, used all compounds.

Torulopsis dattila, culture No. 82, used ammonium oxalate (slightly); l-glutamic acid, hydroxy-l-proline, dl-serine, dl-valine. This fungus refused ammonium acetate and made only slight use of asparagine.

Cryptococcus diffluens, culture No. 83, used all compounds.

Cryptococcus laurentii var. *flavescens*, culture No. 84, used all compounds but calcium nitrate.

Candida pseudotropicalis var. *lactosa*, culture No. 85, used: ammonium chloride, nitrate (slightly), oxalate, sulfate, tartrate (slightly); dl-alpha alanine, dl-histidine HCl, dl-beta phenylalanine, dl-serine, dl-valine.

Cryptococcus laurentii, culture No. 86, used all compounds but calcium nitrate.

Cryptococcus luteolus, culture No. 87, used all compounds but calcium nitrate and magnesium nitrate.

Cryptococcus neoformans, culture No. 88, used all compounds.

Candida pulcherrima, cultures No. 89 and No. 90, used all compounds but sodium nitrate and glycine.

Torulopsis rotundata, culture No. 91, used all compounds.

Torulopsis sphaerica, culture No. 92, used: all ammonium compounds; potassium nitrate; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-threonine (slightly), dl-valine.

Candida utilis, culture No. 93, used all compounds.[2]

Certain refusals are worth mentioning. *Taphrina americana*, *T. caerulescens* from *Q. macrocarpa*, and *T. caerulescens* from *Q. prinoides* refused ammonium chloride, and *T. nana* used it only

[2] Four other fungi were included in the experiments. They were obtained by streak-isolation from material infected with *Taphrina aesculi*, *T. californica*, *T. carnea* (*Betula lutea*) and *T. thomasii*. They resembled known species of *Taphrina* in cellular morphology and in colony characters but they utilized all or nearly all of the compounds offered them. For the present they are believed to be unidentified asporogenous yeasts.

slightly. In a second experiment in which one-half percent agar was included in the medium, ammonium chloride was refused by seven isolates of *T. americana* (an eighth isolated made very slight growth), by eight isolates of *T. caerulescens* from *Q. macrocarpa*, and by six isolates of *T. caerulescens* from *Q. prinoides*. *T. nana* made slight growth (between tr and +).

Ammonium nitrate was refused by *Taphrina nana*, *T. ulmi* from *Ulmus rubra*, *Candida pseudotropicalis* var. *lactosa*, and *Torulopsis sphaerica*.

Of the compounds used less frequently, dl-threonine was utilized only by *T. purpurascens;* beta alanine by *T. communis*, *T. flavorubra*, and *T. purpurascens;* l-cysteine hydrochloride by *T. caerulescens* from *Quercus coccinea*, *Q. macrocarpa*, *Q. prinoides*, *Q. velutina* and by *T. purpurascens*. The compounds just mentioned were used by several of the asporogenous yeasts.

The fact that some fungi refused certain nitrates and used others, although confirmed by repetition of experiments, should, perhaps, not be emphasized. Each nitrate was added to the basal solution without the adjustment necessary to maintain the ion balance. The solution containing magnesium nitrate, for example, was richer in magnesium and poorer in potassium than the one containing potassium nitrate. To the extent that these results furnish information about the nutrition of these fungi it may be said that all organisms but *Taphrina caerulescens* from *Quercus prinoides* and from *Q. macrocarpa* are capable of utilizing nitrate nitrogen. Nevertheless the refusals observed seem to be genuine under the conditions of the experiments and are of diagnostic value.

In applying these data to the characterisation of species of *Taphrina* the following observations may be presented:

All species possess a characteristic pattern of utilization of the various compounds. This is as true for different host-forms of *Taphrina caerulescens* as it is for those fungi recognized as separate species. Previous reports by Thompson (**6**) and by Mix (**3**) have shown that all host-forms of *T. caerulescens* are different morphological entities. The host-forms included in these studies are also different physiologically.

The following host-forms of the same species were found to agree in their utilization of nitrogen compounds: *Taphrina deformans* from *Prunus communis* and from *P. persica; T. robinsoniana* from *Alnus rugosa* and from *A. serrulata; T. sadebeckii* from *Alnus glutinosa* and from *A. serrulata.*

Other host-forms showed differences. *Taphrina betulina* from *Betula intermedia* used six compounds not used by the form from the same host previously known as *T. lapponica* nor by *T. betulina* from *B. pendula.* This last named form differed from the two preceding in refusing ammonium phosphate, sulfate and tartrate, and in using calcium nitrate; otherwise it agreed with "*T. lapponica.*" These three forms, previously known as separate species, were all placed in *Taphrina betulina* by the writer (3) on the basis of similar morphology. The *Taphrina lapponica* of Juel had been distinguished by him because it did not form witches' brooms but affected whole shoots. The writer's observations of this form made in Lappland in 1939 have been previously reported (l.c.). It is possible to find shoots systemically invaded that seem to have been infected for several years without the formation of witches' brooms. Nevertheless it is not possible to tell whether a recently invaded shoot will develop a witches' broom or not. It is apparent that a physiological distinction exists between *Taphrina betulina* Rostr. and *T. lapponica* Juel. Since no morphological differences can be found it seems best to continue to regard them as one species.

Taphrina cerasi from *Prunus pennsylvanica* differed from *T. cerasi* from *P. avium* in refusing hydroxy-l-proline. It also used three compounds refused by the latter fungus: beta alanine, glycine (slightly) and dl-leucine. This fungus on *P. pennsylvanica* was assigned by the writer (3) to *Taphrina cerasi* with some hesitation. It shows no morphological differences from that species. A physiological difference apparently exists.

Of the several host-forms of *Taphrina communis* here studied, some that had been previously distinguished as separate species or varieties were included in this species by the writer (3) because of morphological similarities. It is now apparent that certain physiological differences exist. If the form on *Prunus americana*

is chosen for purposes of comparison these differences can be described as follows: 1. *T. communis* from *P. angustifolia* refused dl-beta phenylalanine and l-proline (used by the preceding) and used in addition l-cysteine hydrochloride and dl-isoleucine (slightly). 2. *T. communis* from *P. lanata* and *T. communis* from *P. nigra* used dl-methionine but otherwise agreed with *T. communis* from *P. americana*. 3. *T. communis* from *P. meritima* used hydroxy-l-proline and dl-methionine (refused by *T. communis* from *P. americana*). 4. *T. communis* from *Prunus* used dl-leucine (refused by *T. communis* from *P. americana*).

Taphrina confusa from *Prunus demissa* used six compounds that were refused by *T. confusa* from *P. virginiana:* beta alanine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, l-proline. This is the greatest difference between supposedly related host-forms found in these studies.

Taphrina epiphylla and the form previously known as *T. klebahni* Wieben but placed in *T. epiphylla* by the writer (3) agreed in their behavior.

Taphrina flavorubra from *Prunus besseyi* and from *P. pumila* var. *susquehanae* agreed except that the former used magnesium nitrate and made very slight use of beta alanine.

Taphrina populi-salicis from *Populus fremontii* and from *P. trichocarpa* agreed except for refusal by the latter of calcium and magnesium nitrates.

Taphrina potentillae from *Potentilla canadensis* differed from *T. potentillae* from *P. arguta* in refusing dl-leucine and in using glycine and dl-methionine.

Taphrina ulmi from *Ulmus rubra* differed from *T. ulmi* from *U. alata* in refusing calcium and potassium nitrates, dl-aspartic acid, and dl-serine, and in utilizing two compounds refused by the former: dl-alpha alanine and l-proline.

The three species from *Acer: T. dearnessii, T. letifera* and *T. sacchari,* appear to be distinct physiological entities. Two of these, *T. dearnessii* and *T. letifera,* though occurring on different hosts are morphologically much alike (3).

Of the thirteen species of asporogenous yeasts studied six appear to be omnivorous and cannot be distinguished on the basis of

these experiments. The others showed some degree of selectivity and two of them, *Torulopsis dattila* and *Candida pseudotropicalis* var. *lactosa*, appear to be quite as specialized in their nitrogen-nutrition as many species of *Taphrina*.

Twelve of the asporogenous yeasts used ammonium acetate. Since the medium invariably became less acid during growth it is believed that the carbon of the ammonium acetate was being utilized. All species of *Taphrina* and one yeast, *Torulopsis dattila*, refused this compound.

None of the species or host-forms of *Taphrina* here studied appeared to need the addition of thiamine in order to grow in the solutions employed. In most cases, however, the addition of thiamine resulted in increased growth, and this increase was often considerable. In the case of the amino acids which were not well utilized no increase was evident, perhaps because the method of measuring growth was not sufficiently accurate. Six organisms: *Taphrina confusa* from *Prunus virginiana* var. *demissa*, *T. nana*, *T. populina*, *T. populi-salicis* from *Populus trichocarpa*, *T. prunisubcordalas* and *T. sadebeckii*, showed no benefit from the addition of thiamine to any of the solutions in which they were grown.

SUMMARY

Fifty-seven host-forms, representing twenty-seven species of *Taphrina*, and thirteen asporogenous yeasts were grown in liquid media with different sources of nitrogen as follows: eight ammonium compounds, four nitrates, urea, peptone, asparagine, and seventeen amino acids.

Ammonium chloride was refused by *Taphrina americana* and by *T. caerulescens* from *Quercus macrocarpa* and *Q. prinoides*.

Ammonium nitrates was refused by *Taphrina nana*, *T. ulmi* from *Ulmus rubra*, *Candida pseudotropicalis* var. *lactose* and by *Torulopsis sphaerica*.

Urea and asparagine were used by all the fungi studied. Peptone was refused only by *Taphrina americana*.

All non-sporulating yeasts but *Torulopsis dattila* used ammonium acetate; none of the species of *Taphrina* studied used this compound.

All species of *Taphrina* showed a characteristic pattern of nitrogen utilization. Some host-forms within species agreed closely in behavior while others showed various degrees of difference.

None of the fungi studied required the addition of thiamine to the media used, though in general they made better growth with added thiamine.

Most of the asporogenous yeasts were omnivorous or nearly so with regard to the compounds offered them. *Torulopsis dattila* and *Candida pseudotropicalis* var. *lactosa* were highly selective.

It seems possible to differentiate species of *Taphrina* in culture on the basis of their use or refusal of different compounds of nitrogen.

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THE ASCOSPORIC STAGE OF ASPERGILLUS CITRISPORUS AND RELATED FORMS

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(WITH 5 FIGURES)

An ascosporic stage of certain molds now recognized as species of *Aspergillus* has been known since 1809. In that year Link described as *Eurotium herbariorum* the yellow perithecia commonly encountered on improperly dried herbarium material. Concurrently, he described as *Aspergillus glaucus* the greenish mold (conidial stage) which occurred with these structures. Nearly a half century later, De Bary (**1854**) demonstrated that the *Aspergillus* heads and the *Eurotium* perithecia were borne on a common mycelium, thus relating Link's ascosporic structures to a large group of saprophytic molds for which no sexual stage was known In the years that followed, several additional ascosporic forms with yellow perithecia were described, usually as species of *Eurotium*. Thom and Church (**1926**) grouped these obviously related forms together as the *Aspergillus glaucus* group, an arrangement which has since become generally accepted. Meanwhile, Eidam (**1883**) described *Sterigmatocystis nidulans*, characterized by reddish ascospores, which has become the focal point for recognizing a second group of ascosporic species around *Aspergillus nidulans* (Eidam) Winter. Finally, Wehmer (**1907**) described *A. fischeri*, ascosporic but without doubt closely related to the well-defined species *A. fumigatus* Fresenius. Prior to the current report, all ascosporic molds that produce an *Aspergillus*-type of conidial stage have been referred satisfactorily to one or

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the other of these three groups. It now becomes necessary to recognize as potentially ascosporic that section of the genus to which *A. citrisporus* von Höhnel belongs.

In July 1950, and again in 1951, one of the authors (H.D.T.) isolated from Wisconsin soils an ascosporic *Aspergillus* which produces conidial heads of the general type seen in the *A. tamarii* and *A. flavus-oryzae* groups, and perithecia which are at first parenchymatous and resemble developing sclerotia but subsequently become filled with large and conspicuously sculptured ascospores. Ascocarps of this type are markedly different from those produced in the *A. glaucus*, *A. nidulans*, or *A. fumigatus* groups. It seemed obvious that we were dealing with an undescribed species belonging to some group wherein ascospores had not been reported previously.

In checking carefully the species recognized by Thom and Church (**1921**, **1926**), reference was found to a culture obtained by them from Thaxter under the unpublished name *Aspergillus chrysospermus* which he had verbally reported to produce ascospores. Insofar as we have been able to determine, this observation was never reported in the literature; and no description of the species was ever published. In their publication of 1921, Thom and Church report "sclerotia occasionally produced" when the mold was recultivated in their laboratory. Essentially the same statement was repeated in "The Aspergilli," published five years later. Yet, in Thom's culture notes dated May 20, 1919, the following notations appear with reference to Thaxter's culture: "Perithecia, 500 μ—soft, easily crushed. Asci 12–14 μ diam." Ascospores were not observed.

Upon the basis of their examination of the conidial structures of Thaxter's fungus and von Höhnel's brief, but seemingly well-prepared description of *A. citrisporus*, Thom and Church concluded that the fungi observed by von Höhnel and Thaxter represented a single species. Accordingly, they accepted von Höhnel's usage, recognized *A. citrisporus* as the valid species name, and distributed Thaxter's isolate to various laboratories, including the Centraalbureau in Baarn, as *A. citrisporus*, strain No. *1186.10*.

When cultivated at the Northern Regional Laboratory and compared with known species of the *A. tamarii* group, the similarity between Tresner's isolates and Thom and Church's concept of *A. citrisporus* became clearly evident. We then obtained Thaxter's exsiccati (labeled *A. chrysospermus*) from the late Professor W. Lawrence White, Curator of the Farlow Herbarium, in order to compare the ascospores of his material with those produced by the new isolates from Wisconsin soil. Also, we secured from the Centraalbureau, through the courtesy of Dr. Johanna Westerdijk, the culture of *A. citrisporus* which had been deposited by Thom and Church in March 1925 and which stemmed originally from Thaxter's laboratory. In our hands this latter culture has failed to produce ascospores, although a great variety of culture media has been investigated. Fortunately, we found abundant perithecia and ascospores in Thaxter's herbarium specimens. In this material, as in the growing cultures of Tresner's isolates, the perithecia were characterized by a marked purplish coloration and superficially resembled the sclerotia formed in many species of *Aspergillus*. The ascospores, however, were strikingly different from those produced in the Wisconsin isolates. This circumstance, together with certain other differences of lesser significance, has led us to conclude that two species are involved, and that each merits recognition as an ascosporic species. This necessitates (1) the redescription of *A. citrisporus* to include a perfect stage, exemplified by Thaxter's exsiccati and the still living culture (conidial phase only) which stems therefrom, and (2) the description of a new species, *Aspergillus ornatus,* to include the Wisconsin strains.

SPECIES RECOGNIZED

ASPERGILLUS CITRISPORUS von Höhnel emend.

Colonies on Czapek's solution agar restricted and thin, attaining a diameter of 1.0 cm in two weeks at room temperature (FIG. 1A), consisting of a sparse, submerged growth of vegetative hyphae, and bearing few small conidial structures with conidiophores seldom exceeding 500 μ in length or vesicles 15 μ in diameter.

Colonies on malt extract agar growing rapidly, attaining a diameter of 8–10 cm in 10 days at room temperature, confluent, heavily

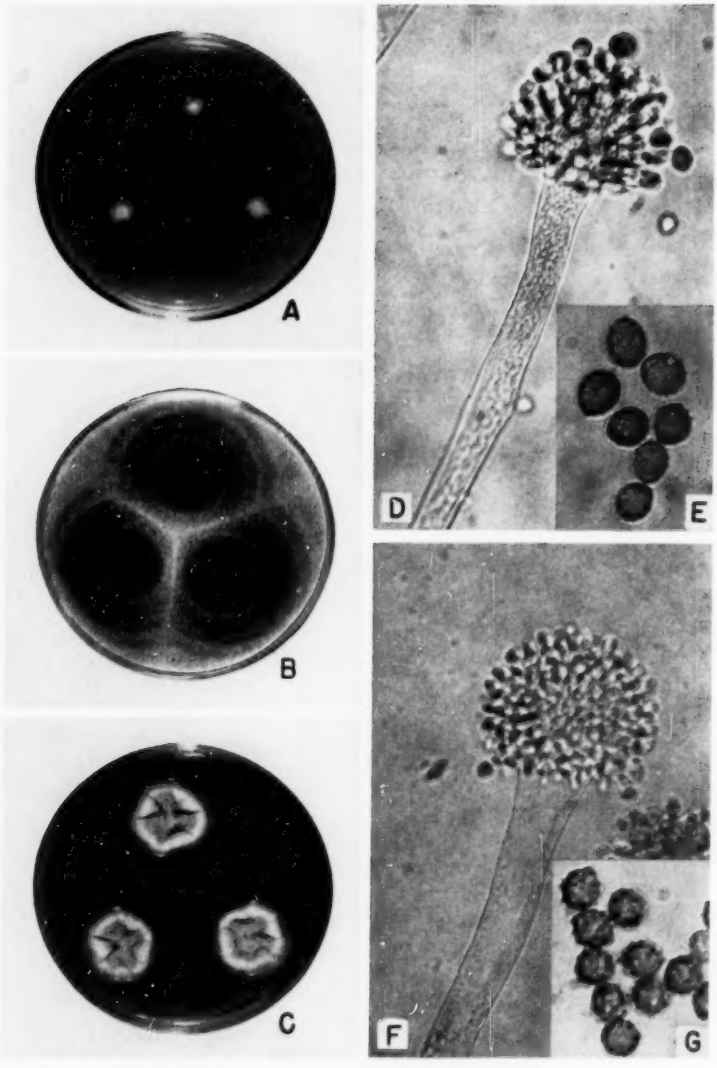

FIG. 1. *Aspergillus citrisporus*. Conidial phase. A, B, and C. Cultures growing on Czapek's solution agar (with sucrose), malt extract agar, and Czapek's agar with glucose, respectively. 10 days incubation in diffuse daylight at room temperature. D. Conidial head of NRRL *2292*, which strain stems from one of Thaxter's collections (see FIG. *2* and text) through Thom's No. *4186.10*, × 450. E. Mature conidia of the same, × 1000. F. Conidial head of NRRL *2293* (Warcup's isolate), × 450. G. Mature conidia of the same strain, × 1000.

and uniformly sporulating throughout with conidial structures arising from submerged vegetative mycelia to form a dense stand (FIG. 1B); incubation in darkness not restrictive; conidial heads in orange-yellow shades, at first near aniline yellow, becoming orange citrine at maturity, and darkening to medal bronze in age (Ridgway, IV); reverse uncolored; odor pronounced, somewhat foetid. Conidial heads radiate, mostly 150–200 μ, but ranging from 100 to 225 μ, with conidial chains loosely divergent. Conidiophores variable, commonly 1.5–2.0 mm in length by 15–20 μ in diameter above, septate, with walls comparatively thick (*ca.* 1.0–1.5 μ at median level), smooth as seen in fluid mounts but appearing granular when observed dry. Vesicles somewhat flask-shaped, 20–30 μ diameter with uppermost three-fourths or entire surface bearing sterigmata in a single series (FIG. 1D), often loosely arranged. Sterigmata variable, generally 8–12 $\times$ 5.5–7.0 μ. Conidia citriform or pyriform to subglobose, light yellow-brown, conspicuously roughened, mostly 6.0–7.5 $\times$ 5.0–6.0 μ (FIG. 1E).

Colonies on potato dextrose agar essentially like those produced on malt extract agar but growing somewhat more thinly and producing a less dense stand of conidiophores.

Colonies on Czapek's solution agar containing glucose instead of sucrose growing somewhat restricted (FIG. 1C), forming a dense mycelial felt somewhat wrinkled and furrowed, and producing abundant but generally diminutive conidial structures.

Perithecia (seen in Thaxter's exsiccati only) globose or nearly so, variable in size but commonly 300–500 μ in diameter, in purple shades, with or without an enveloping network of much-branched, coarse mycelia, upon crushing appearing somewhat parenchymatous but well filled with ascospores; perithecial wall comparatively thin at maturity and composed of polyhedral cells 10–12 μ diameter (FIG. 2E); asci with nearly mature spores measure about 12 μ diameter (FIG. 2C); ascospores lenticular, smooth-walled, 7.5–8.5 $\times$ 5.0–6.0 μ, with single (?) broad equatorial bands (FIG. 2C, F).

The emended description of *Aspergillus citrisporus* von Höhnel here presented is based upon a culture, NRRL *2292*, bearing this name which was received from the Centraalbureau voor Schimmelcultures in June, 1951, together with Thaxter's herbarium specimens obtained from the Farlow Herbarium of Cryptogamic Botany, Harvard University. There is ample reason to believe that the former culture stems directly from the latter, and that we are, in fact, drawing the description of conidial and ascosporic

stages from the same original isolation, albeit the connecting links are somewhat devious and extend over more than a quarter century. The story is as follows: The culture of *A. citrisporus* received from the Centraalbureau is listed as having come from Thom and Church. Records of these latter investigators indicate that a fungus bearing the numerical designation *H186.10* was sent to Baarn in March, 1925, under this name. This culture, cited

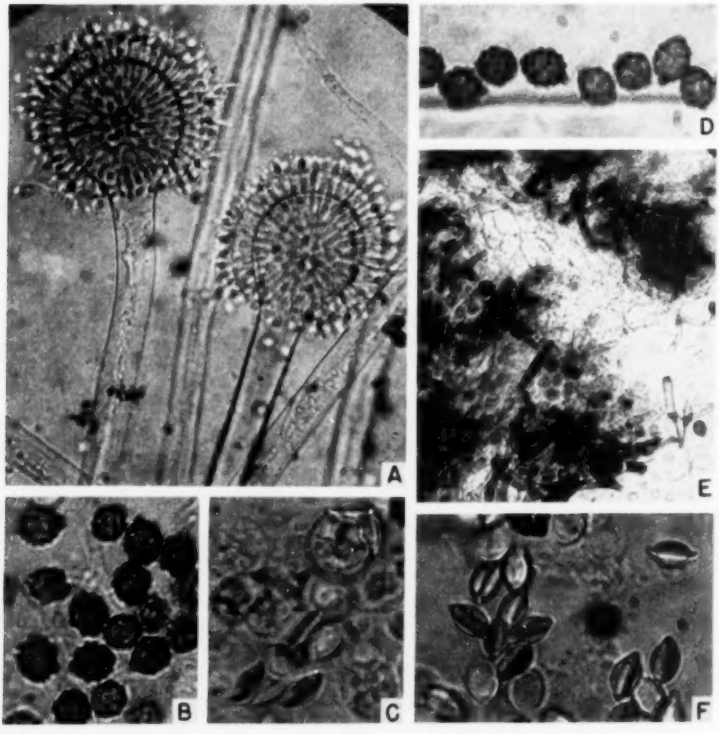

FIG. 2. *Aspergillus citrisporus:* Thaxter's herbarium material. A. Conidial structures from his dried culture (medium not stated) made from the "Chocoura, N. H." collection, ×275. B. Mature conidia from the same, ×850. C. Asci and ascospores from the same culture, ×850. D. Mature conidia from a dried culture of the "Kittery Point" collection, ×850. E. Portion of perithecial wall from same culture, showing hexagonal peridial cells and fragments of branched enveloping hyphae, ×275. F. Mature ascospores from the same, ×850.

by Thom and Church (**1926**) as representative of *A. citrisporus*, had been obtained from Thaxter as "*Aspergillus chrysospermus*" in March, 1917, and entered in Thom's Collection under the number indicated above. At this point an element of uncertainty enters our chain of evidence, although it in no way invalidates the claim of culture continuity from Thaxter's laboratory to our own. Among Thaxter's material are two groups of specimens, both of which (1) represent isolates from caterpillar dung; (2) bear the designation *A. chrysospermus;* (3) show conidial heads with single sterigmata (FIG. 2A) and large, coarsely roughened, citriform conidia (FIG. 2B, D); and (4) contain purple, parenchymatous perithecia with large, smooth-walled ascospores which are marked by single broad equatorial bands (FIG. 2C, F). One of the two is labeled "Chocorua, N. H., Aug., 1909" (FIG. 2A, C); the other is marked "Kittery Point" and bears no collection date (FIG. 2D, F). Whereas it is possible that the culture given to Thom in 1917 may have come from either source, it is believed probable that the Kittery Point collection is of more recent date and that NRRL *2292* stems from it. The point is not too important, in any case; for insofar as we can determine the two collections are strikingly similar.

A third collection of Thaxter's labeled "Hammond's Pond, Mass., Sept. 17, 1902," is preserved only as a permanent slide mount and shows no ascosporic stage, hence is not believed to represent a possible progenitor of NRRL *2292*. The few conidial heads that are present in this specimen, however, agree closely with the other two.

One additional culture, NRRL *2293*, which we believe to represent the species under consideration, was isolated from New Zealand forest soil and sent to us in January, 1948, by Dr. J. H. Warcup of the Botany School, Cambridge University, as "*A. citrisporus* von Höhnel (?)." This produces heavily sporulating colonies on malt extract agar and on other natural substrata of this type. The conidiophores are appreciably shorter than those cited above, but in other particulars it agrees reasonably well with the species as here described (FIG. 1F, G). In aging cultures (3–6 weeks) on malt extract agar, colonies commonly de-

velop white mycelial tufts which superficially suggest the perithecia seen in Thaxter's specimens and regularly produced in growing culture of *A. ornatus*. Upon closer examination these are found to consist of rather densely interwoven hyphal threads rather than parenchymatous tissue, and no asci or ascospores have been observed. Despite their absence, we feel that these structures probably represent abortive perithecia, and it is hoped that some combination of substratum and cultural conditions can be found which will permit their full development. At such time only can the strain in question be diagnosed more satisfactorily.

Aspergillus ornatus n. sp.

Coloniae in agaro Czapekii tarde crescentes, tenues, pellucidae, fructificationibus conidicis ex hyphis hyalinis submersis vegetis oriundis, peritheciis carentibus, in agaro maltoso luxuriantes, frutificationibus conidicis ex hyphis submersis oriundis et super stratum superficialem peritheciorum protrudentibus, late zonatae vel sectores efformantes, praecipue flavo-aurantiacae, e primulino aureae usque olivaceo-citrinae, reverso non colorato; capitulis conidicis radiatis, plerumque 300–400 μ in diam.; conidiophoris septatis 1.0–1.5 mm longis, 12–18 μ in diam.; membranis comparate crassis, humidis glabris, sed aridis subasperatis; vesiculis lageniformibus, per quartas tres superiores fertilibus, plerumque 25–40 μ in diam.; sterigmatibus uniseriatis, confertis, plerumque 8–10 $\times$ 4.5–5.5 μ; conidiis e aureis pallide brunneis, citriformibus vel pyriformibus, e liris irregularibus vel lineis coloratis crasse asperatis, plurimum 6.5–8.0 $\times$ 5.0–6.0 μ; peritheciis in agaro maltoso abundantibus, primum albis parenchymaticisque, in maturitate purpurascentibus, parte fertili peridio cellularum irregularium polyedricarum circumdata; ascis globosis, 14–15 μ; ascosporis lenticularibus, asperatis, cristis multiplicibus tenuibus 1 μ latis irregulariter sed vulgo longitudinaliter dispositis ornatis.

In culturis e solo in silvis, Wisconsin.

Colonies on Czapek's solution agar growing very sparingly, 2.0–2.5 cm in 10 days at room temperature, thin, transparent (FIG. 3A), with scattered small conidial structures arising from submerged and colorless vegetative hyphae; conidial heads radiate, commonly 150–200 μ in diameter and borne on conidiophores seldom more than 650 μ in length. No perithecia produced.

Colonies on malt extract agar growing luxuriantly, attaining a diameter of 7.0–8.0 cm in 10 days at room temperature (FIG. 3B), with vegetative mycelium largely submerged or superficial, broadly zonate from alternating bands of crowded and less numerous conidial structures projecting above the surface layer of abundant perithecia, less commonly tending to sector with some areas pre-

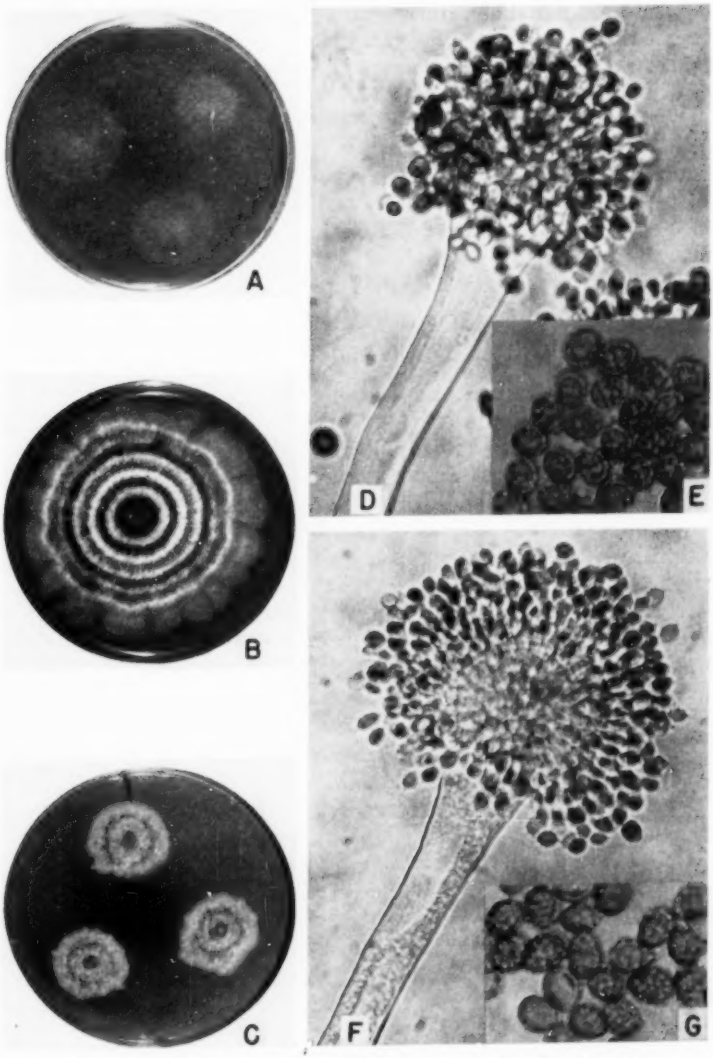

FIG. 3. *Aspergillus ornatus*. Conidial phase. A, B, and C. Cultures of type strain NRRL *2256* growing upon Czapek's solution agar (with sucrose), malt extract agar, and Czapek's agar with glucose, respectively. 10 days incubation in diffuse light at room temperature. D. Conidial head in type strain, × 525. E. Mature conidia of the same, × 1000. F. Conidial head in strain NRRL *2291* (Tresner's second isolate), × 450. G. Mature conidia of the same, × 1000.

dominantly conidial and others heavily perithecial; conidial structures arising from submerged hyphae, abundantly produced in light, lacking or sparingly developed in darkness, in yellow-orange shades from primuline yellow when young through old gold to olive-citrine in age (Ridgway, XVI); heads radiate, with conidial chains divergent and somewhat tangled, variable in size, commonly 300–400 μ in diameter but ranging from 200–450 μ; conidiophores septate, commonly 1.0–1.5 mm in length by 12–18 μ diameter above, somewhat narrowed below, with walls comparatively heavy (*ca.* 1.0–1.5 μ at a median level), appearing smooth in fluid mounts but somewhat roughened when examined dry; vesicles flask-shaped, mostly 25–40 μ diameter, fertile over the upper three-fourths, bearing crowded sterigmata in a single series; sterigmata crowded, mostly 8–10 $\times$ 4.5–5.5 μ; conidia golden to light brown, mostly citriform to pyriform, coarsely roughened from an irregular network of ridges or color bars, commonly 6.5–8.0 $\times$ 5.0–6.0 μ. Perithecia abundantly produced, variable in size but commonly 400–600 μ diameter, at first white and parenchymatous throughout, becoming purple and filled with asci and ascospores at maturity (FIG. 4C), the fertile portion remaining surrounded by a residual peridium of irregular polyhedral cells 15–20 μ in diameter (FIG. 4D) within a loose mantle of sterile, much-branched hyphae; asci globose, 14–15 μ at maturity; ascospores lenticular, rough-walled, with multiple thin, flexuous crests approx. 1.0 μ wide arranged irregularly in a generally longitudinal direction, over-all dimensions generally 7.5–8.5 $\times$ 5.5–6.0 μ (FIG. 4E).

Colonies on potato dextrose agar spreading broadly, 8–9 cm in 10 days, comparatively thin, especially in marginal areas; conidial heads abundant but not crowded, averaging about 250 μ in diameter, and near old gold (Ridgway, XVI) when mature; perithecia abundantly produced, averaging somewhat smaller than on malt extract agar.

Colonies on Czapek's solution agar with glucose instead of sucrose growing somewhat restrictedly, 2.0–2.5 cm in 10 days (FIG. 3C), with compact basal mycelium more or less wrinkled producing numerous conidial heads, thinning toward the irregular colony margin; perithecia occasionally developed in old cultures.

The binomial *Aspergillus ornatus* is chosen because of the extraordinary ornamentation of the ascospores.

Type culture NRRL *2256* was isolated by one of the authors (H.D.T.) from soil collected in an oak woods just west of the city of Madison, Dane County, Wisconsin (7N–9E–29), in July, 1950.

A second strain (NRRL *2291*), which duplicates the type in all essential particulars, was subsequently isolated from soil collected in a maple woods in Spring Grove Township, Greene County, Wisconsin (1N-9E-29). Both soils were taken from the "A"

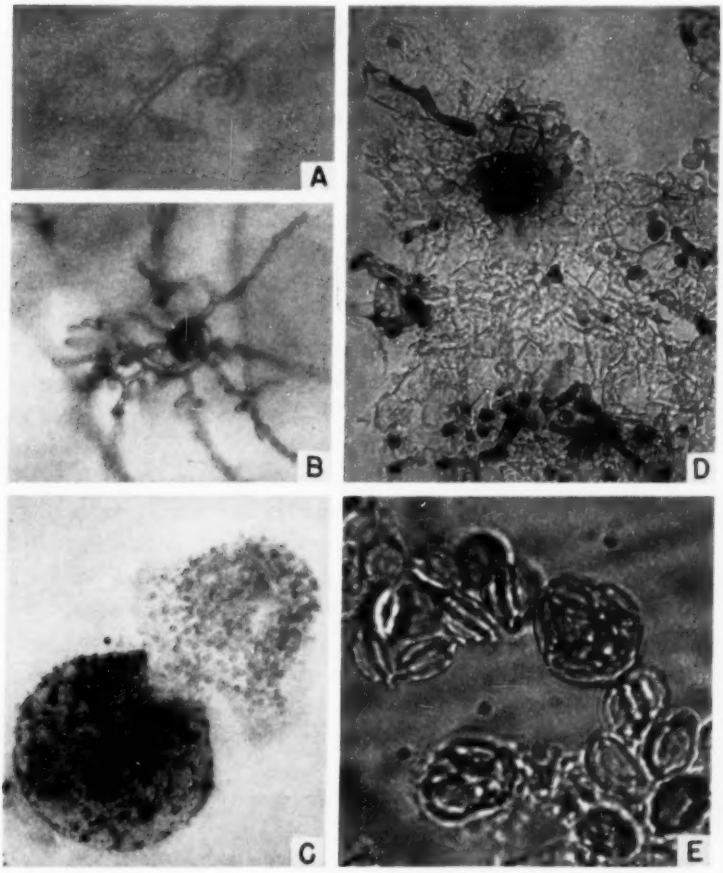

FIG. 4. *Aspergillus ornatus:* NRRL *2256*. Ascosporic phase. A. Young ascogonium, × 175. B. Somewhat later stage, × 175. C. Mature perithecium, crushed to expose ascigerous tissue, × 65. D. A portion of the perithecial wall showing the hexagonal cells and remnants of the coarse branched hyphae which comprise it, × 325. E. Mature ascospores showing characteristic ornamentation, × 1000.

horizon, i.e., the top 1½ inches. The writers are indebted to Professor Myron P. Backus, University of Wisconsin, for having made the preliminary examination of this fungus, for recognizing the unusual characteristics of its ascosporic phase, and for bringing it to our attention in order that we might study it critically.

GROUP RELATIONSHIPS

Aspergillus citrisporus von Höhnel emend. and *A. ornatus* represent a new departure in ascosporic species of *Aspergillus*. For several reasons they cannot be placed in any of the ascosporic groups previously recognized. Like members of the *A. glaucus* group, they produce conidial heads that are radiate in pattern and bear a single series of sterigmata; but, unlike these forms, they produce conidiophores which appear roughened when viewed in dry mounts. Most importantly, they develop purple, parenchymatous perithecia that are markedly different from the consistently yellow, thin-walled structures seen in *all* ascosporic species of the *A. glaucus* group. They cannot be closely related to *Aspergillus nidulans* (Eidam) Wint. and allied forms, for they do not develop Hülle cells or show colored stalk walls. Additionally, they produce colorless ascospores in contrast to the reddish spores that are so characteristic of the *A. nidulans* group. They obviously do not belong with *A. fischeri* Wehmer, for their conidial heads are much larger and are radiate rather than columnar. As with the preceding groups, the most striking difference consists in the character and texture of their ascocarps as contrasted with those of *A. fischeri*.

Whereas the origin of perithecia cannot be established for *A. citrisporus* from currently available material, in *A. ornatus* perithecia arise from coiled ascogonia (FIG. 4A, B) that are strikingly different from the initially unbranched primordia observed in other ascosporic groups. Additionally, ascospores seem not to germinate by valvular separation as in other Aspergilli, but by simple swelling and the production of germ tubes as in the conidia of this species and in the ascospores of many Penicillia.

Superficially, colonies and conidial structures of *A. citrisporus* and *A. ornatus*, when grown upon favorable media such as malt

extract agar, bear a striking resemblance to those of some members of the *A. tamarii*, *A. wentii*, and *A. flavus-oryzae* groups. Furthermore, immature perithecia of the two former species are strongly suggestive of the developing sclerotia that characterize the latter groups of asexual Aspergilli. Weighing such facts, together with differences in the perithecial stage of the presently reported ascosporic species as compared with perfect forms long verified, we are led to the conclusion that a fourth group must be recognized as potentially ascigerous.

It is perhaps too early to determine just what realignments should be made within the genus *Aspergillus*. Obviously, the arrangement of groups and species worked out by Thom and Church (**1926**) and, in the main, restated by Thom and Raper (**1945**), will have to be altered. The schematic diagram of group relationships presented by the latter (**1945**, p. 87) will have to be redrawn, placing greater or lesser emphasis upon some of the characters which heretofore have been used effectively to differentiate groups of species that are clearly related. Based upon present knowledge, and the comparative examination of cultures presently available, it would seem desirable to regard the *A. tamarii* group as an ascigerous one, recognizing, as we do in the *A. glaucus*, *A. nidulans*, and *A. fumigatus* groups, that all member species need not attain this level of development. In time it may be found desirable to transfer the species *A. tamarii* Kita to the *A. flavus-oryzae* group, with which it shares many characteristics in common, and to recognize a new group of species centering upon some ascosporic form such as *A. citrisporus* or *A. ornatus* and to include with it *A. lutescens* (Bain.) Thom and Church, *A. terricola* Marchal, and *A. terricola* var. *americana* Marchal. This would bring together a fairly homogeneous group of species characterized by loose-textured, radiate conidial heads with sterigmata in a single series, except for the larger structures in *A. lutescens*.

The precise natural substratum of *A. ornatus* is not known. In the case of *A. citrisporus*, however, all known isolates, with the exception of NRRL *2293* from Warcup, have been obtained from the dung of various insect larvae. It is easily possible that such material actually supported Warcup's isolate as well as the two

isolates of *A. ornatus*. On the other hand, all of these forms may occur occasionally in soil; and the high incidence of *A. citrisporus* on caterpillar dung may merely reflect inoculation from such source, coupled with the subsequent collection of dung pellets as discreet entities.

PHYSIOLOGICAL STUDIES

In preliminary cultural investigations performed at the University of Wisconsin it was observed that the isolate (NRRL *2256*) which now constitutes the type of *Aspergillus ornatus* grew very thinly, sporulated sparsely, and produced no perithecia upon Czapek's solution agar; whereas it grew luxuriantly and produced abundant conidial heads and perithecia on malt extract and potato-dextrose agars. This observation was immediately confirmed at the Northern Regional Research Laboratory upon receipt of the culture from Professor Backus. Furthermore, it was soon noted that in plate cultures on Czapek's solution agar, *A. ornatus* grew well and sporulated freely in portions of colonies adjacent to contaminating molds. Thus it was obvious that the culture suffered from some nutritional deficiency which was partially or wholly corrected by the contaminant.

Two possibilities immediately suggested themselves: (1) A need for some essential vitamin, or (2) an inability to utilize sucrose.

To test the first of these possibilities, vitamins of the B-complex were added in excess *via* cylinder cups to cultures of *A. ornatus* growing upon Czapek's solution agar plates. Doubtfully significant responses were obtained with riboflavin (B_2) and cobalamin (B_{12}), whereas no measurable response was obtained with thiamin, inositol, para-amino benzoic acid, pyridoxine, pyridoxal, biotin, folic acid, nicotinic acid, and pantothenic acid. Since vitamins B_2 and B_{12} were added as strongly acidic solutions (pH 2.8 and 1.5, respectively), the possible effect of pH could not be ruled out; and subsequent tests tended to minimize the possibility of a true nutritional response from either vitamin.

Meanwhile, other experiments pointed to an inability to utilize sucrose as an increasingly probable explanation. For example, it

was observed that *A. ornatus* attained satisfactory, but not luxuriant, growth on Czapek's solution agar containing glucose instead of sucrose and on Czapek's solution agar with sucrose as a carbon source but with KH_2PO_4 substituted for K_2HPO_4. Since it was known that sucrose when heat sterilized in the presence of KH_2PO_4 would be partially hydrolyzed, it was thought that the mold might be growing only upon the hexoses present in the latter medium, as it was of necessity doing in the former. By analysis, Czapek's solution agar with initial concentrations of 3% sucrose and 0.1% KH_2PO_4 showed from 0.3 to 0.4% reducing sugar and from 2.6 to 2.7% sucrose after heat sterilization. Interestingly enough, the growth of *A. ornatus* on such media was not obviously greater than on Czapek's solution agar containing 0.3% glucose as sole carbon source and 0.1% KH_2PO_4, where both the sugar and the phosphate were added as Seitz filtrates. In other experiments Seitz-filtered sucrose was added to sugar-free Czapek's solution agars of varying pH, and in no case did growth exceed a very thin, submerged mycelium bearing few and diminutive conidial heads. Obviously, sucrose could not be utilized by *A. ornatus;* but reducing sugars, particularly glucose, seemed to afford an acceptable carbon source. Further tests indicated that this constituted only a partial answer. An unanticipated response was encountered when Seitz-filtered glucose was similarly added to sugar-free agar media of different pH. Good growth was initiated rapidly in acidic media (pH 5.0–5.5), whereas for a period of several days growth was minimal on media of slightly alkaline reaction (pH 7.5 ±). By the end of two weeks, however, growth was proceeding satisfactorily in the latter, and the reaction of the substrate adjacent to the growing colonies had been depressed to pH 4.5 ±, which at this age approached the reaction attained by cultures growing in the initially acidic media. From the above, and other substantiating experiments, we conclude that glucose constitutes a favorable carbon source for *A. ornatus*. It becomes optimally effective, however, only in the presence of an acidic environment. This may be established prior to inoculation by the introduction of acid phosphate, or it may be created by the growing fungus itself by the accumulation of acidic products which

result from the metabolism of glucose. The sugar availability *vs.* acidity relationship seems to be even more striking in the case of fructose. This sugar (as a Seitz filtrate) affords a satisfactory carbon source for *A. ornatus* when added to acidic (pH 5.5 ±), carbon-free agar media. On the other hand, growth hardly exceeds that obtained with sucrose on comparable media that are at first neutral or slightly alkaline, and the pH is not significantly depressed, even after two weeks incubation.

Whereas these nutritional investigations have centered around *A. ornatus* NRRL *2256*, the other strain of this species and those of *A. citrisporus* already mentioned have been included quite often for comparison. This has been especially true of NRRL *2292*, the culture of *A. citrisporus* supplied by the Centraalbureau. Allowing for normal species and strain variation among closely related forms, these other cultures behave in a manner strikingly similar to NRRL *2256*. In every case, growth is luxuriant upon malt extract agar and is minimal upon Czapek's solution agar containing sucrose as a carbon source, irrespective of pH. In each case satisfactory growth is obtained with glucose as carbon source in media which are initially acidic. Strain NRRL *2292* differs from the others in being less able to grow satisfactorily on glucose media of initial neutral reaction even after extended incubation.

The production of conidia and perithecia by *Aspergillus ornatus* NRRL *2256* is strongly affected by light. Initial experiments were conducted in the Botany Department, University of Wisconsin, by M. Louis Bobichon and others. Concerning these studies, Professor Paul J. Allen writes as follows (personal communication):

"This [light] effect was studied particularly on [an agar] medium containing 4% of an extract of blended woody twigs, which permits growth at half the maximum rate obtained with honey-peptone agar. In complete darkness no conidia are formed on this medium, while in continuous white light of low intensity heavy sporulation occurs. Illumination by a spectrum projected across a plate which had been uniformly inoculated with spores resulted in sporulation in blue light. A few minutes' illumination of mycelium which has grown in the dark caused heavy sporula-

tion only at the margin of the colony and not on the older parts. Marked zonation is therefore exhibited by colonies grown in alternating light and dark.

"Light distinctly inhibits perithecia formation, but does not suppress it entirely."

Some additional investigations were made at the Northern Regional Laboratory. For the most part, these were comparative studies wherein strain NRRL *2256* was contrasted with the second isolate of *A. ornatus*, NRRL *2291*, and with the two strain assigned to *A. citrisporus* NRRL *2292* and *2293*. Observations

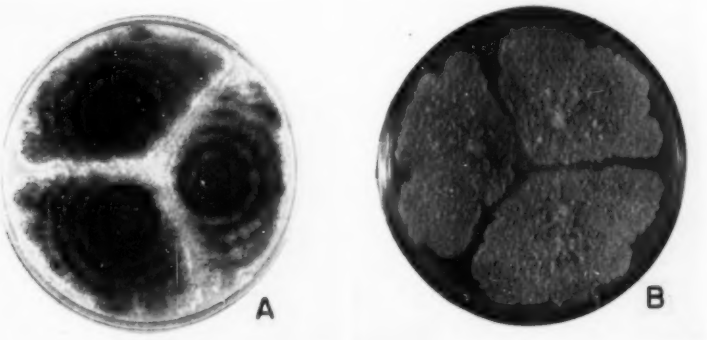

FIG. 5. Effect of light upon the production of conidial structures in *Aspergillus ornatus* NRRL *2256*. Colonies on malt extract agar, 10 days incubation at room temperature. A. Culture incubated in diffuse daylight. B. Culture incubated in darkness.

made on NRRL *2256* confirmed, in general, those reported from Wisconsin. On the majority of media investigated, colonies produced very few or no conidial heads when incubated in darkness, whereas such structures were produced abundantly in parallel cultures incubated in diffuse daylight (FIG. 5A, B). Czapek's solution agar enriched with 1% corn steep liquor provided a striking exception: On this medium conidial production in darkness approached the level attained in light. Incubation temperatures ranged between 24–27° C but were uniform for light- and dark-incubated cultures.

Aspergillus citrisporus NRRL *2292* and *2293* showed a response markedly different from *A. ornatus* NRRL *2256*. Neither of the former strains showed any apparent difference in conidial production whether the cultures were incubated in darkness or in light. *A. ornatus* NRRL *2291* was somewhat intermediate in behavior, conidial production being sharply reduced but not suppressed in dark-incubated cultures.

Obviously, the effect of light on sporulation represents a strain characteristic. Nevertheless, it may prove valuable in differentiating between *A. ornatus* and *A. citrisporus*. The observation that the effect of darkness can be tempered in NRRL *2256* by modifying the substrate suggests some interesting possibilities for further investigation. What is contained in steep liquor that enables the mold to circumvent, in large measure, the metabolic block which on other substrata effectively precludes the development of conidial structures in darkness?

DISCUSSION

A number of considerations led us to associate the Wisconsin isolates with *Aspergillus citrisporus* von Höhnel. Among these was the general aspect of growing colonies on a variety of substrata, together with the color and pattern of the conidial structures which are characterized by radiate heads bearing sterigmata in a single series. Whereas conidial heads of this structural type are regularly seen in certain members of the *Aspergillus flavus-oryzae* group, e.g., *A. parasiticus* Speare and some cultures of *A. oryzae* (Ahlburg) Cohn, a golden to light olive-brown coloration of conidial heads and the absence of consistently rough stalks in the Wisconsin strains indicated closer relationship with members of the *A. tamarii* group (see p. 683). Criteria which pointed more specifically to *A. citrisporus* were found in descriptions of this species published by Thom and Church (**1921**, **1925**), and more particularly in Thom's unpublished cultural notes. In these the meagre growth of the species on Czapek's solution agar was emphasized, and some attention was given to the "sclerotia" (perithecia) which were produced in Thaxter's culture shortly after its receipt. Altogether, the conidial phase of the Wisconsin

isolates seemed to fit reasonably well the species concept for *A. citrisporus* von Höhnel as this had been interpreted, enlarged, and presented by Thom and Church (**1921**).

With a feeling of confidence that our presumptive diagnosis would be confirmed, we requested Professor White to lend us Thaxter's collections representing *A. citrisporus,* or *A. chrysospermus,* the unpublished name used by Thaxter. In particular, we wished to obtain the specific collection from which Thom's No. *4186.10* stemmed, for this culture had formed the principal basis for accreditation of *A. citrisporus.* Furthermore, it had been deposited with the Centraalbureau, and for twenty-five years it had been distributed from that Collection under this name. As expected, the conidial structures in Thaxter's materials, after reconstitution, were generally similar to those in the Wisconsin strains. Furthermore, the perithecia in these dried specimens were strikingly similar in color and texture to those regularly produced in growing cultures of the new isolates. The ascospores, however, were markedly different (FIG. 2C, F, 4E). It was at first difficult to believe that the spores were not basically similar, and that apparent differences could not be attributed to thoroughly dry material in the one case as contrasted with living material in the other. The observed differences were accepted as real, however, when recognized techniques for swelling and reconstituting fungus tissues failed to reveal any method of treatment whereby the ascospores appeared similar.

One may question the wisdom of combining, in a single species description, observations taken from living cultures on the one hand and from long dried herbarium materials on the other. In the case of *A. citrisporus* we believe this to be justified, for we can trace the two contributory elements to a common source with reasonable certainty. Such treatment, of course, does not take into account possible variations in the living culture which has for a quarter of a century been grown on laboratory substrata. Furthermore, there is evidence that our accepted culture of *A. citrisporus,* NRRL *2292,* has changed somewhat during this period. The conidia would seem to be less coarsely roughened, heads and vesicles would seem to be somewhat smaller, and, of

course, the capacity to produce perithecia and ascospores has been lost. Admittedly, our approach is not entirely satisfactory. On the other hand, if we were to prepare the complete diagnosis from exsiccati, we would perforce exclude such characteristics as color, rate of growth, and texture and appearance of the growing fungus —features which are most important in identifying species of *Aspergillus*.

One may question, also, whether *A. ornatus* merits recognition as a new and separate species. Certainly, the conidial stages are strikingly similar in this species and in *A. citrisporus*, as we interpret the latter. Furthermore, the perithecia are quite similar in the two cases. Such parallelism, however, does not invalidate species recognition; in fact, the situation is strictly comparable to accepted practice in the *A. glaucus* and *A. nidulans* groups, where species are recognized primarily upon marked and consistent difference in ascospore patterns.

Aside from morphological similarities, the generally meagre growth of *A. citrisporus* and *A. ornatus* on synthetic media containing sucrose as a carbon source serves additionally to indicate their close relationship. A like response has been reported for *A. panamensis* (Raper and Thom, **1944**). There, as here, the paucity of fungus growth is presumed to stem from an invertase deficiency. This limiting deficiency can be alleviated in each case by substituting glucose for sucrose. However, in the case of *A. ornatus* and *A. citrisporus* a further restrictive condition is imposed, and glucose should be supplied in an acid environment to attain a maximal response.

The discovery of an ascosporic stage in a species of *Aspergillus* assigned to a group wherein sclerotia have long been known, coupled with the fact that the perithecia themselves are at first parenchymatous and structurally resemble sclerotia, naturally raises the question, "What other species now regarded as sclerotium-producers may contain ascosporic strains?" Following a precedent established by Charles Thom, we have for years examined the sclerotia in newly isolated strains of *A. wentii*, *A. flavus*, and many other sclerotium-producers in the hope of finding an ascosporic form. In *Penicillium* the line separating

sclerotial and perithecial forms is thin indeed. Our search among the Aspergilli, like Thom's before us, has been in vain. Extension of our search for additional forms, and the introduction of new procedures for isolating them, may in time justify our optimistic hope. The possibility should not be dismissed lightly.

SUMMARY

Two additional species of *Aspergillus* are recognized as ascosporic, namely, *Aspergillus citrisporus* von Höhnel emend. and *A. ornatus* n. sp. Both species grow luxuriantly upon malt extract agar and other nutrient-rich natural substrata. Neither species grows well upon Czapek's solution agar with sucrose as a carbon source, but each grows satisfactorily when glucose is substituted for sucrose and the medium is rendered mildly acidic. Based upon cultural characteristics and upon details of morphology in the conidial phase, these species seem to belong near to or within the *A. tamarii* group. The perithecia in both species are strikingly similar and are markedly different from fertile structures previously reported in the genus *Aspergillus*. They are parenchymatous at first, become purplish at maturity, and superficially resemble the young sclerotia of various Aspergilli. These newly recognized ascosporic species cannot be placed in the *A. glaucus*, *A. nidulans*, or *A. fumigatus* groups, long established as ascigerous sections of the genus *Aspergillus*. It becomes necessary, therefore, to recognize a fourth group of species as potentially ascosporic. For the present it seems that such can best be aligned with the *A. tamarii* group.

ACKNOWLEDGMENTS

The writers wish to express their indebtedness to Professor Myron P. Backus, University of Wisconsin, for first recognizing and calling to our attention the unusual characteristics of *A. ornatus;* to the late Professor W. Lawrence White, Farlow Herbarium, for making Professor Thaxter's collections available to us; to Dr. Johanna Westerdijk, Centraalbureau voor Schimmelcultures, for returning to us a living culture of the strain of *A. citrisporus* which had been sent to her by Thom and Church, and

which was subsequently lost from our Collection; to Dr. Charles Thom, U. S. Department of Agriculture (retired), for personal consultation and access to cultural notes; to Edith K. Cash, Division of Mycology and Disease Survey, USDA, for preparing the Latin diagnosis of *A. ornatus;* and to Messrs. George E. N. Nelson and Roland Haines of the Northern Regional Laboratory for making sugar determinations and preparing illustrative material, respectively.

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A CLEISTOTHECIUM-LIKE STRUCTURE FOUND IN HORMODENDRUM PEDROSOI

H. Karrer and N. F. Conant

(WITH 1 FIGURE)

One of the etiologic agents of chromoblastomycosis, *Hormodendrum Pedrosoi,* is known to reproduce in culture by at least three types of imperfect sporulation (**9**). Among the many strains which have been isolated, the lack of or the predominance of one or another of these types of sporulation also is known to occur (**3**). Such polymorphism exhibited by this fungus has made its exact taxonomic position a controversial subject for many years with the result that it has been placed in the following genera: *Acrotheca* (Fonseca and Leão, 1923); *Trichosporium* (Ota, 1928); *Gomphinaria* (Dodge, 1935); *Botrytiodes* (Moore and Almeida, 1936); *Phialoconidiophora* (Moore and Almeida, 1936); *Hormodendroides* (Moore and Almeida, 1936); *Fonsecaea* (Negroni, 1936); *Phialophora* (Emmons, 1944).

It has been suggested that this taxonomic problem can be resolved only when the perfect stage of *H. Pedrosoi* becomes known (**4**). Recently an advance has been made in this direction with a report of structures resembling immature cleistothecia in one strain of *Phialophora* (**1**). A similar structure also found in one strain of *H. Pedrosoi* will be the subject of this report.

MATERIALS AND METHODS

Sabouraud's glucose agar (4% Difco glucose, 1% Difco neopeptone, 2% agar), corn-meal agar (Quaker Oats Co., yellow enriched corn meal) as prepared by Benham (**2**) and corn-meal agar fortified with 1% Difco yeast extract were used in this study. The fungus was grown on the above media in Petri dishes, in tubed slants and on slide cultures as described by Riddell (**11**).

One particular strain of *H. Pedrosoi,* used in this laboratory for teaching purposes for a number of years, was found to produce cleistothecia-like bodies. Its original number has been lost, however, so that it cannot be identified as to original source. Numerous other strains of *H. Pedrosoi* were grown either separately or in combination with the above strain for the purpose of testing heterothalism and/or cross fertility.

RESULTS

The *Hormodendrum, Acrotheca* and *Phialophora*-type of sporulations were produced on all of the media by this strain of *H. Pedrosoi* (FIG. 1, e, f, g). Cleistothecia-like bodies reached their greatest number, largest dimension and more perfect development, however, on Sabouraud's glucose agar slide cultures at room temperature and could be examined microscopically from permanent stained preparations of such cultures (FIG. 1, a). They were observed to develop within two weeks as lateral outgrowths from the mycelium. They were greenish in color at first but became progressively darker with age. From small multicelled structures arising laterally from the mycelium and resembling multicellular chlamydospores (FIG. 1, d), they developed into large round, membranous to carbonaceous, cleistothecia-like bodies, 40 μ in size, with a parenchymic wall of one layer of polygonal cells, from the base of which arose many septate setae of varying length (FIG. 1, b, c).

Such bodies could also be observed on slide cultures of corn-meal 1% yeast-extract agar but they were less well developed, being smaller, more irregularly shaped and often lacking setae. On plain corn-meal agar slide cultures they were nearly always absent.

In Petri dish cultures the bodies were seen only occasionally when Sabouraud's glucose agar was used, but not at all on plates of corn-meal yeast-extract agar. They were not found on any of the above media in agar slant cultures.

Several attempts to demonstrate asci and ascospores within the cleistothecia-like structures were unsuccessful. Fully developed bodies were crushed in lactophenol-cotton blue only to yield

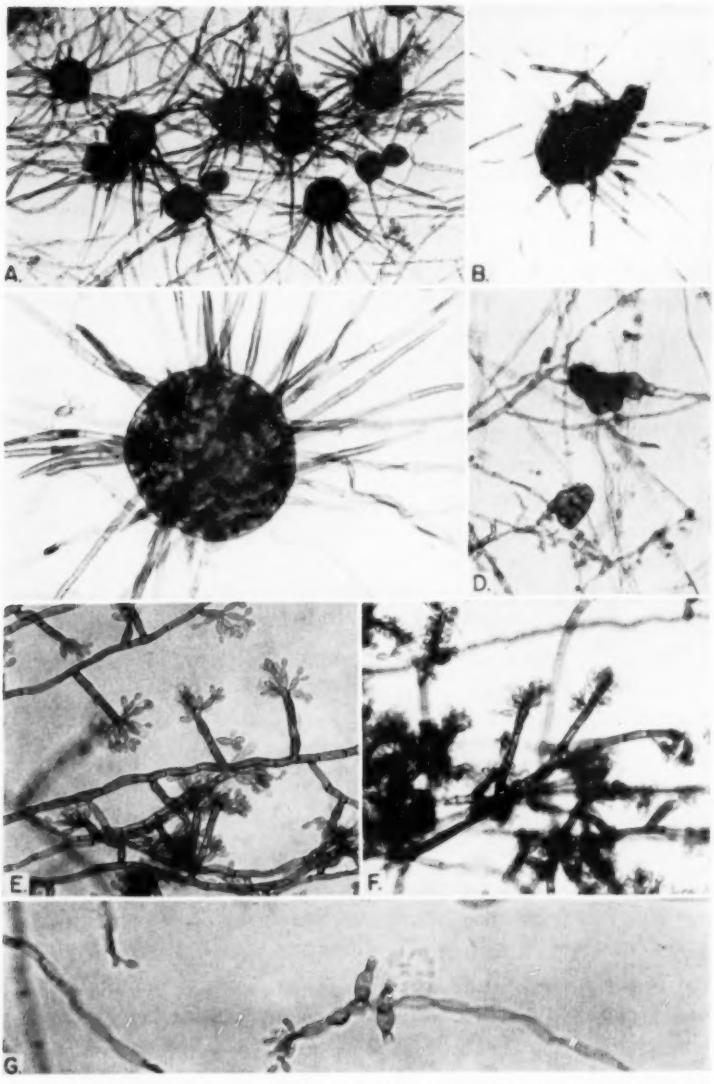

FIG. 1. *Hormodendrum Pedrosoi*. All photomicrographs taken from permanent preparations of Sabouraud's glucose agar slide cultures. A. Cluster of cleistothecium-like structures, × 254. B. Single beaked structure, × 350. C. Typical cleistothecium-like structure with setae, × 720. D. Small multicelled chlamydospore (sclerotium-like) bodies, × 350. E. Typical *Hormodendrum*-type sporulation, × 650. F. Typical *Acrotheca*-type sporulation, × 650. G. Typical *Phialophora*-type sporulation, × 870.

fragments of deeply stained parenchymous cells. Although such fragments superficially resembled asci with deeply stained internal structures, careful examination revealed their true nature.

Numerous other strains of *H. Pedrosoi* failed to develop cleistothecia-like structures when grown on the same media and under the conditions described above. An attempt to demonstrate heterothalism and/or cross fertility among these strains was not successful. To date, no strain or combination of strains grown in the neighborhood of the one forming cleistothecia-like structures, either in microcultures or on plates, has been able to stimulate the formation of mature ascus-bearing structures.

DISCUSSION

The morphology of the structure described for this one strain of *H. Pedrosoi* resembles exactly that found by Ajello (**1**) in one strain of *Phialophora verrucosa*. It is strongly suggestive of an immature ascocarp. Smaller, younger bodies which may be called chlamydospore-like, or sclerotia, have been observed previously by Carrión (**5**) and Carrión and Silva (**7**). The lack of asci may be due to the absence, under the conditions studied, of physical or chemical factors necessary for maturity or to the absence of proper strains to complete fertilization by one means or another.

After the discovery of a *Phialophora* type of sporulation in strains of *H. Pedrosoi* (**6**), it was suggested that the phialid-borne conidia might serve as spermatia (**8**, **10**). In previous investigations, and in the present studies, however, attempts to cross-fertilize strains of *H. edrosoi* have not been successful.

The report of similar cleistothecia-like structures in strains of two fungi which cause chromoblastomycosis draws attention to the possibility that this group of fungi might ultimately be classified in a single genus of the Ascomycetes. This does not imply, however, that the classification of the imperfect stages, *Phialophora* and *Hormodendrum*, will be affected. In the first place, the imperfect generic name will carry over as, for example, *Aspergillus* for the imperfect stage of *Eurotium*. In the second place, the ultimate perfect stages of these fungi may be found to represent different species in the same genus each with its own, *Phialophora* or *Hormodendrum*, imperfect stage. An example of

such a situation recently has been described by Whitehead and Dickson (**12**) in which *Pyrenophora alternaria* was shown to have an *Alternaria* imperfect conidial stage and *P. secalis* to have a *Helminthosporium* imperfect conidial stage. It may still be necessary, therefore, for taxonomists to resolve the difficulties inherent in the taxonomy of the imperfect fungi causing chromoblastomycosis by means other than the discovery of their perfect stage.

SUMMARY

A new structure found in one strain of *Hormodendrum Pedrosoi* is described. The possibility of its representing an immature ascocarp is discussed.

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COMPARATIVE STUDIES OF FOUR RHIZOCTONIAS OCCURRING IN LOUISIANA

BEATRICE EXNER

(WITH 9 FIGURES)

Among the species of *Rhizoctonia* which have been reported in Louisiana there are four of particular interest from a pathological standpoint. These are responsible for diseases of considerable importance on certain crops. They are the ones which have been reported in the literature as *Corticium microsclerotia* Weber (*Rhizoctonia microsclerotia* Matz), the cause of web-blight of beans and other plants, *Corticium sasakii* Matsumoto, the cause of the banded scleroital disease of sugarcane, rice and certain grasses, the ubiquitous *Corticium solani* (Prill. and Del.) Bourd. and Galz. (*Rhizoctonia solani* Kuhn), the cause of root and stem diseases of many plants, and an undescribed *Corticium* which has been reported in Louisiana (**16**) as causing a leaf blight of figs.

The relationship and taxonomy of these four Rhizoctonias has been a problem among plant pathologists. Before the change in 1943 to *Pellicularia* by Rogers (**13**), *Corticium* had been accepted as the name of the genus to which the four fungi belonged, and each was considered a separate species by the workers who described the perfect stages. However, similarities observed among the basidiomycetous stages and certain cultural characters of single basidiospore isolates as reviewed or reported in this paper have thrown doubt on the validity of these species.

In preliminary studies (**14**, **1**), it was found that a rather large number of cultural types occurred among single basidiospore isolates of these fungi. In 1921 Muller (**11**) found that basidiospore cultures of the fungus known as *Rhizoctonia solani* differed in certain characters and suggested that segregation of types probably occurred in the formation of basidiospores. It was felt that

further comparative studies of the cultural characters of tissue isolates, the basidiomycetous stages, and variations among single basidiospore cultures would reveal something of their relationship. Also, the single basidiospore cultures offered a means of studying the possible role that the basidiomycetous stages play in the origin of physiological races which have been reported by various investigators for *Rhizoctonia solani* (e.g., **15**, **5**) and *Corticium sasakii* (**7**).

In order that these fungi may be referred to separately without synonyms, the names used in the first part of this paper up to the discussion will be *Corticium solani*, *Corticium microsclerotia*, *Corticium sasakii*, and *Corticium* sp.

MATERIALS AND METHODS

The diseases caused by the four fungi can usually be distinguished by the symptoms produced on the various hosts which they attack. This was determined by isolations from the diseased hosts and the identification of the fungus found associated with the disease. Small pieces of diseased tissue were placed on water agar on which the pathogen soon outgrew all other organisms, and pure cultures were obtained from the leading edges of the mycelia. For identification the cultures were compared with representative cultures obtained from stock cultures at Louisiana Agricultural Experiment Station. Among the stock cultures was a culture of *Corticium sasakii* Matsumoto which had originally come from Matsumoto, a culture of *C. miscrosclerotia* Weber which had been obtained from Weber, and cultures of the fig blight organism obtained by Tims and Mills, who first reported the disease (**16**).

Descriptions of typical tissue isolates were made from cultures grown on potato dextrose agar (250 gms Irish potatoes, 20 gms dextrose, 15 gms agar, sufficient water to make 1000 cc) for two weeks at 28° C. Selected typical cultures were later grown on Czapek's agar under the same conditions to compare formation of sclerotia on this synthetic medium.

In periods of heavy rainfall and high humidity in June, July, and August, the basidiomycetous stages of these fungi have been

found in abundance on various hosts in Louisiana. Frequently extensive hymenia were found on plants which were not being parasitized. The fungi were merely growing over the surface and forming the basidial stage. Basidial mats from 14 hosts were examined and identified by the cultures arising from basidiospores or by cultures which came from plating out pieces of the basidial mats. Length and width measurements were made on 3285 basidiospores in mounts made from 62 collections. Basidia with fully extended sterigmata were rarely seen; however, at least ten from each of the four fungi were found and measured in length and width.

Several hundred single basidiospore cultures were isolated for comparative studies of the cultural characters and pathogenicity to determine the extent of variation within each group. These cultures were obtained by isolating germinating spores scattered over the surface of potato dextrose agar of about 1 mm depth in Petri dishes. Small strips of basidial mats were made to adhere to wet filter paper in the covers of sterile dishes containing the agar. The covers were moved around periodically to distribute the spores over the surface of the agar. After the spores had begun to germinate, isolated spores were marked, the agar was cut with a "biscut cutter," 1.5 mm in diameter, after the manner described by Keitt (**4**), and the pieces were transferred to sterile dishes, where they were examined to be certain that only one spore occurred on each piece. The single spores were then transferred to tubes of agar.

The cultures used in comparisons were grown under the same conditions as nearly as possible. They were first compared in tubes of potato dextrose agar, and the various strains found among the isolates were then transferred to plates of potato dextrose agar. After 48 hours growth, three uniform pieces of inoculum were cut with a 5 mm cork borer and transferred to triplicate plates, each containing 25 cc of potato dextrose agar. The agar used in each experiment was from a single batch.

The rate of growth was determined by measuring the diameters of the mycelia incubated at 28° C at the 24th and 48th hour. The difference between the two measurements was regarded as the increase in diameter per 24-hour period.

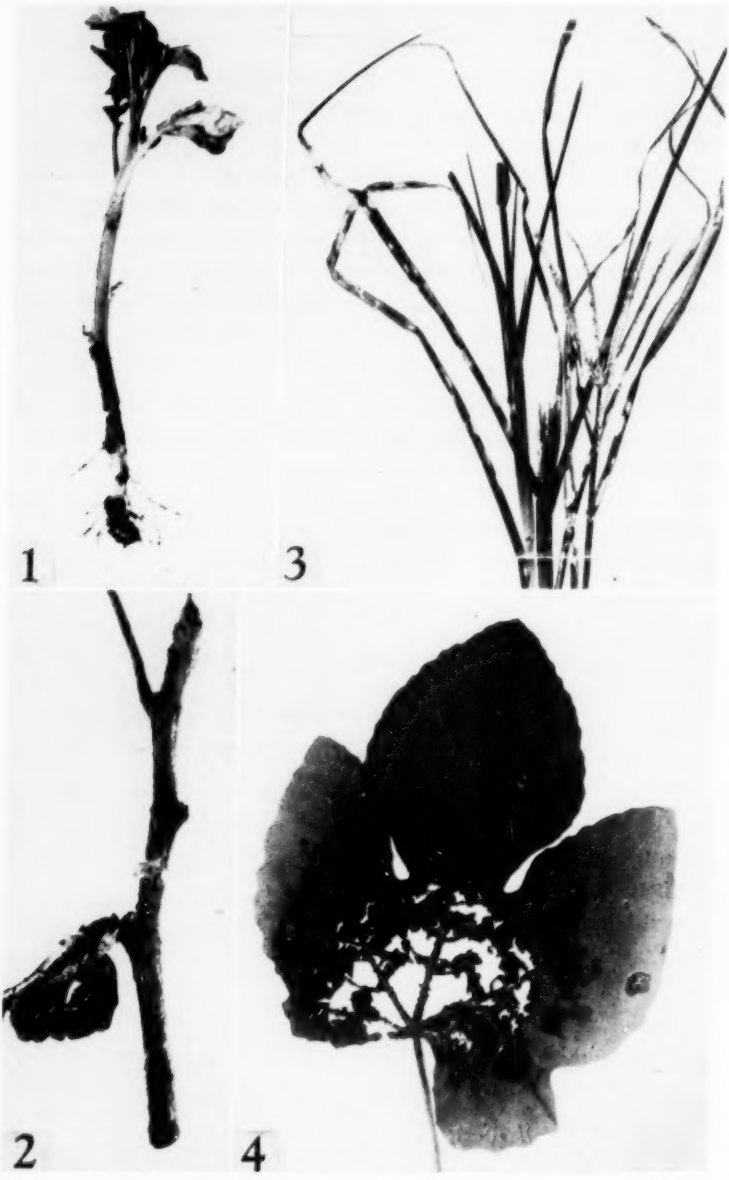
After two weeks growth at this same temperature the cultures were examined for other cultural differences—size, shape, number, and arrangement of sclerotia; color and topography of the mycelium; and ability of the cultures to darken the medium. The extent to which the cultures could be classified on the basis of descriptions of typical tissue isolates was determined and tabulated.

To determine differences in pathogenicity, bush snap beans were grown as the test host in sterilized soil artifically infested with a number of the isolates. The inoculum was prepared by growing the isolates for a week on a sterile oat-wheat mixture (four parts oats and one part wheat to which an equivalent weight of water was added). Two pots were used for each isolate. Twenty seeds to the pot were planted in the infected soil and watered from day to day. After 26 days the plants were removed from the pots, and the soil was washed away from the lower stems and roots so that the extent of injury could be determined. To measure the pathogenicity, an arbitrary scale of infection similar to the one described by Person (**12**) was used. Each plant was examined to determine the severity of lesions formed and then placed in one of the four following infection classes:

0 = no infection
1 = small lesions on hypocotyl and roots
2 = large lesions on hypocotyl and roots
3 = dead or dying plants

The index of pathogenicity was determined for each isolate by multiplying the number of plants in each group by the number assigned to that infection class and dividing the sum of the products by the total number of plants. A pathogenicity index of 1 would be moderately pathogenic, and of 2 or over would be severely pathogenic.

Ten cultures from a basidial mat of *Corticium solani* were tested as to damping off, using cabbage, tomato, and egg plant as test hosts. Three pots of sterilized soil were inoculated with each culture for each host, and 50 seeds were planted in each pot. The counts on the number of seedlings damped off were taken periodically for 18 days.



FIGS. 1–4.

SYMPTOMS OF THE DISEASES

Root and stem rots of various hosts (FIG. 1). This is the common group of diseases caused by the fungus known as *Rhizoctonia solani*. The symptoms are usually a rotting of the roots and cankers on the stems near the soil line. If the plants are very young, damping off may occur.

Web-blight (FIG. 2). This disease is characterized by a necrosis of the leaves and stems of various plants. The primary diognostic character is the occurrence of numerous small brown sclerotia from 0.2 to 0.5 mm in diameter, which look somewhat like grains of sand scattered over the surface of the host. Blighted leaves cling to other leaves or to stems, and when they are pulled apart it is seen that they are held together by cobweb-like threads. This disease was first reported in 1916 from Florida by Matz (**8, 9**) who named the causal organism *Rhizoctonia microsclerotia*. In 1940 Weber (**17, 18**) found basidial mats on bush snap beans affected with web-blight and named the organism *Corticium microsclerotia*.

This disease is known to occur on a large number of plants, but it has been found most frequently in Louisiana on three hosts—snap bean (*Phascolus vulgaris*), lima bean (*Phascolus lunatus*), and fig (*Ficus carica*).

Banded sclerotial disease (Fig. 3). This disease occurs most frequently on members of the family Gramineae, although it has been found on snap bean (*Phascolus vulgaris*), lima bean (*Phascolus lunatus*), *Dichondra repens*, peanut (*Arachis hypogea*), Alyce clover (*Allysicarpus vaginalis*), and *Commelina* species. The type of injury is distinct in that large, irregular, bleached spots occur in the leaves and sheaths. Sometimes a banded effect is produced on grass blades when the spots are numerous. Globoid sclerotia, 2–5 mm in diameter, are often produced on the diseased plants. The roots apparently are not affected, and the stems of

FIGS. 1–4. Disease symptoms produced by four Rhizoctonias: 1. Root and stem rot on young potato, caused by *Corticium solani*. 2. Web-blight on Celeste fig, caused by *C. microsclerotia*. 3. Banded sclerotial disease of grasses on *Eleusine indica*, caused by *C. sasakii*. 4. Leaf blight on Celeste fig, caused by *C.* sp.

diseased plants are only occasionally injured. The fungus causing this disease is evidently the same as the one called *Corticium sasakii* by Matsumoto (**6**) in 1934.

A leaf blight of figs (FIG. 4). This disease is similar in appearance to web-blight of figs caused by *Corticium microsclerotia* Weber and thread blight caused by *Pellicularia koleroga* Cooke. However, it can be distinguished from web-blight by the fact that no microsclerotia are formed on the host and from thread blight in that no mycelial threads and no flat, crusty sclerotia, characteristic of the thread blight fungus, are produced. This disease is caused by an undetermined *Corticium* and was first reported by Tims and Mills (**16**) in 1938.

One of the four fungi listed above, only *Corticium solani* has been isolated in nature from roots or lesions on basal stems. This indicated that in nature the other three fungi were confined to aerial parts of hosts attacked.

CULTURAL CHARACTERS OF THE FOUR RHIZOCTONIAS

When tissue isolates were made from the diseased material of the four diseases, a rather distinctive cultural form was found to be associated with each disease, but microscopic examination of the mycelia of the four forms revealed no characteristic by which one could be distinguished from the others. After two weeks growth on potato dextrose agar and Czapek's agar, they all possessed to a varying extent the short-celled, stout hypha, called sclerotial cells, which attained a diameter of 10–18 μ and had formed characteristic sclerotia in cultures of *Corticium microsclerotia, C. sasakii,* and the undetermined *Corticium.*

Although several tissue isolates were found to be almost identical in cultural characters with each of the representative cultures, some variation in cultural characters was observed among cultures in each group. However, the differences were so slight that classification was not difficult. The variations observed among cultures of *Corticium solani* were similar to those reported by Person (**12**), Houston (**2**), Kotila (**5**), and other workers, and the variations observed among cultures of *C. sasakii* were similar to those reported by Matsumoto, Yamamoto and Hirane (**7**).

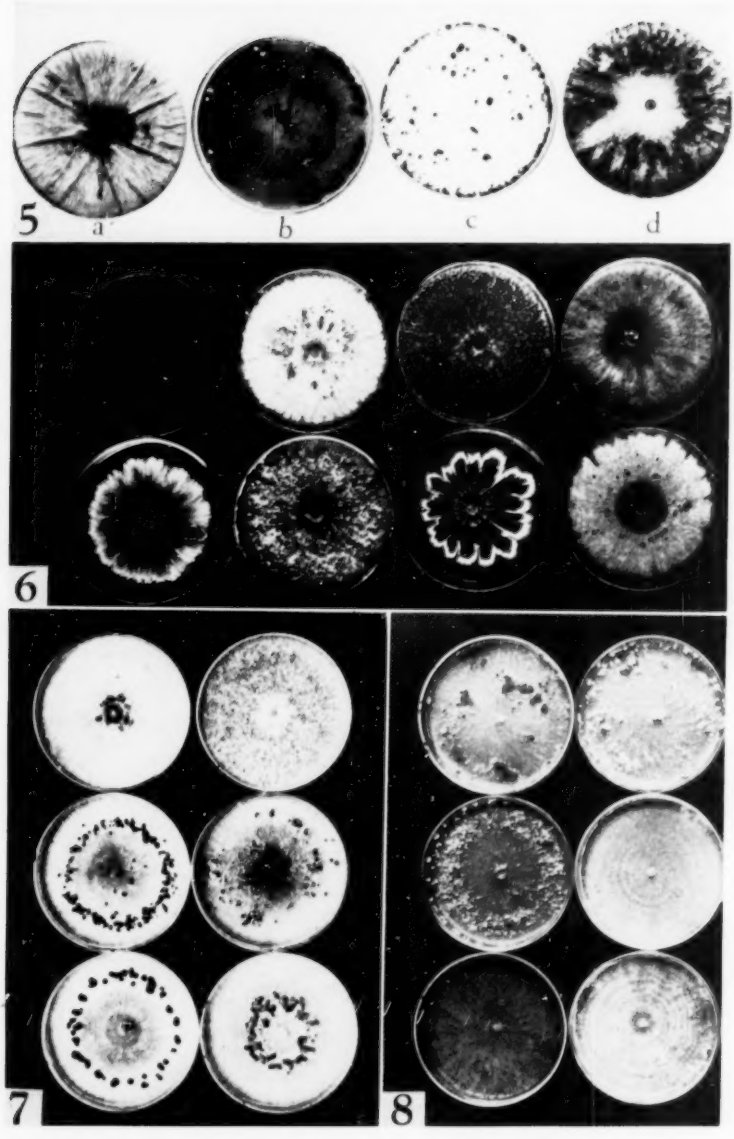
All cultures made from diseased plants covered with microsclerotia could easily be identified as *C. microsclerotia* and all cultures made from severely blighted fig leaves on branches devoid of sclerotia could easily be recognized as the undetermined *Corticium*. Formation of sclerotia by *C. solani*, *C. microsclerotia* and *C. sasakii* was practically the same on Czapek's agar as on potato dextrose agar. The undetermined *Corticium* produced a greater number of separate sclerotia which could be easily measured on Czapek's agar. FIG. 5 shows the four fungi grown for two weeks on potato dextrose agar.

The following descriptons were made from tissue isolates:

Corticium solani. The cultures in this category were variable in the production of sclerotial bodies, but were easily distinguished from the other three Rhizoctonias, which produced characteristic sclerotia. Some of the cultures produced small, irregular sclerotia scattered over the surface of the agar; some produced flattened coalescent sclerotia, usually in the center of the plate; and others produced radiating lines or thin patches of sclerotial cells on the surface of the agar.

Corticium microsclerotia Weber. Although the cultures in this group produced numerous, very small, grayish-brown sclerotia on the host, on media they produced few but very large sclerotia, which were tawny in color when young and deep brown at maturity. The sclerotia varied in size from 3–7 mm in width, from 4–11 mm in length, and 2–3 mm in depth. The average from 2 representative cultures was 4.4 mm in width, by 6.1 mm in length, by 2.6 mm in depth. The largest sclerotia, which were slightly irregular in shape, were usually the result of coalescence of two to four smaller sclerotia.

The fungus causing web-blight in Louisiana was evidently the same as the one described in 1939 by Weber (**17**, **18**) in Florida, but a different strain from the one described by Matz (**10**) in 1921. The one which Matz called *Rhizoctonia microsclerotia* produced small sclerotia on both host and agar. Weber's *Corticium microsclerotia* produced small sclerotia on the host and large sclerotia on agar and therefore fitted more closely Matz's description of a strain which he called *R. dimorpha* (**10**).



FIGS. 5–8.

Corticium sasakii Matsumoto. The cultures in this group were characterized by the production of sclerotia on the agar which were rather definite in shape and size. They appeared spherical to ovoid from the top, but were slightly flattened underneath. They varied in size from 1–5 mm in width, from 1–6 mm in length, and from 1–2 mm in depth. The average for the sclerotia measured from nine isolates was 2.0 mm in width, by 2.6 mm in length, by 1.6 mm in depth. The sclerotia were first light colored and became deep brown, almost black, as they became older.

The undetermined Corticium. These cultures produced a large number of flattened, irregular sclerotia which coalesced to form rough, crusty aggregations over a large part of the surface of the agar and on the sides of the petri dish, when grown on potato dextrose agar. The sclerotial aggregations were grayish-brown to deep brown in color. On Czapek's agar many separate sclerotia were formed. They were round to elongate, ranging in size from 0.5–7 mm in diameter and 0.5–4 mm in depth, deep brown underneath with a lighter colored cottony top.

THE BASIDIOMYCETOUS STAGES

No distinguishing character was found by which the basidial mats of the four fungi could be separated. They appeared as thin, powdery, white layers over the surfaces of stems and leaves and became beige as they grew older.

The hymenial layer in each case was made up of a loose weft of articulate hyphae from which the basidia arose in small, imperfect cymes. The stalk cells apparently formed branch hyphae from which new basidia were developed.

The basidia were similar and agreed with descriptions given by various authors. They were barrel shaped or clavate, bearing four sterigmata (occasionally two) which arose as blunt knobs and later became horn-shaped.

FIGS. 5-8. 5. Typical tissue isolates of four Rhizoctonias on potato dextrose agar: a. *Corticium solani;* b. *C. microsclerotia;* c. *C. sasakii;* d. *C.* sp. 6-8. Single basidiospore cultures showing variations in cultural characteristics of isolates from a single hymenium: 6. from *C. solani;* 7. from *C. sasakii;* 8. from *C. microsclerotia.*

The basidia and sterigmata were variable within a single collection and the ranges of the measurements were overlapping among the four fungi. The basidia of *Corticium microsclerotia* ranged from 6.8–13.6 μ in length, averaging 10.6; 5.4–9.4 μ in width, averaging 7.1 μ; and the sterigmata ranged from 6.8–10.2 μ in length, averaging 8.7 μ. The basidia of *Corticium sasakii* ranged from 9.0–14.0 μ in length, averaging 12.4 μ; 7.0–10.0 μ in width, averaging 8.3 μ; and the sterigmata ranged from 5.0–9.0 μ in length, averaging 7.7 μ. The basidia of *Corticium solani* ranged from 10.2–17.0 μ in length, averaging 14.0 μ; 7.0–10.0 μ in width, averaging 8.8 μ; and the sterigmata ranged from 13.0–16.0 μ in length, averaging 14.2 μ. The basidia of the undetermined *Corticium* on fig ranged from 9.8–14.7 μ in length, averaging 12.2 μ; 6.5–9.8 μ in width, averaging 8.3 μ; and the sterigmata ranged from 6.5–12.2 μ in length, averaging 8.5 μ.

TABLE I

MEASUREMENTS OF BASIDIOSPORES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.) FROM BASIDIAL MATS FORMED IN NATURE

	No. basidial mats	No. hosts	No. spores measured	Average in μ		Range in μ	
				Width	Length	Width	Length
C. solani	9	3	825	5.31	8.93	4.99–5.63	8.55–9.20
C. microsclerotia	12	5	583	5.15	8.30	4.70–5.78	7.68–9.44
C. sasakii	21	10	763	5.20	8.46	4.67–5.92	7.36–9.49
C. sp.	20	1	1114	5.52	8.54	4.67–6.24	7.55–9.47
Totals	62	19	3285				

The basidiospores produced by the four Rhizoctonias were found to be indistinguishable from one another. They were ellipsoid or oblong-ellipsoid, smooth, thin-walled, flattened on the side toward the center of the group, and truncate-apiculate.

The results of measurements of the large number of basidiospores taken from the 62 collections are given in TABLE I. The average dimensions of 583 basidiospores from 12 collections of hymenia formed by *Corticium microsclerotia* were 5.15 × 8.3 μ, of 763 basidiospores from 21 collections of hymenia formed by *Corticium sasakii* were 5.2 × 8.46 μ, of 825 basidiospores from 9 col-

TABLE II

INCREASE IN DIAMETER IN MM FROM 24TH TO 48TH HR ON POTATO DEXTROSE AGAR AT 28° C OF 269 SINGLE BASIDIOSPORE CULTURES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.)

Basidial mat	No. cultures giving increases in diameter of					Total cultures
	0–10 mm	11–20 mm	21–30 mm	31–40 mm	41–50 mm	
C. solani						
A	0	0	4	2	0	6
B	0	0	8	13	0	21
C	1	1	9	9	1	21
D	0	1	5	17	1	24
E	1	3	5	5	1	15
F	1	4	4	10	0	19
G	0	0	5	7	1	13
J	0	0	8	19	2	29
C. microsclerotia						
I	1	1	14	3	0	19
M	4	1	1	10	4	20
C. sasakii						
S	0	1	4	10	10	25
C. sp.						
O	16	17	3	3	0	39
F	4	11	3	0	0	18

lections of *Corticium solani* were 5.31 × 8.93 μ, and of 1114 basidiospores from 20 collections of the undetermined *Corticium* on fig were 5.52 × 8.54 μ.

VARIATION AMONG SINGLE BASIDIOSPORE CULTURES

Corticium solani. In a previous note by Exner and Chilton (**1**) it was reported that comparative cultural studies showed a large number of distinct types among the progenies from single basidiospore cultures from 9 basidial mats from various hosts. As many as 29 distinct cultural types were found among the cultures from one small section of a basidial mat. These strains showed wide differences in their rate of growth, also. Further isolations made

after that showed the same occurrence of cultural differences (TABLE III) and differences in rate of growth among cultures from a basidial mat (TABLE II). However, all the cultures could be identified as belonging to *Corticium solani*, as it is differentiated from the other three Rhizoctonias in these studies (TABLE III).

TABLE III

CULTURAL STRAINS SECURED FROM SINGLE BASIDIOSPORE ISOLATES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.)

Basidial mat	Host plant	No. of cultures isolated	No. of cultures compared in plates	No. of cultural strains	No. of cultures identifiable as same as parental type
		C. solani			
A	Lima bean	7	6	6	6
B	Lima bean	24	21	21	21
C	Lima bean	52	21	21	21
D	Lima bean	83	24	24	24
E	Alligator weed	60	15	15	15
F	Alligator weed	30	19	19	19
G	Irish potato	57	13	13	13
J	Irish potato	75	29	29	29
Q	Alligator weed	27	7	7	7
W	Alligator weed	6	5	5	5
Z	Alligator weed	16	8	8	8
X	Alligator weed	15	9	9	9
i	Alligator weed	38	35	20	20
j	Alligator weed	32	27	17	17
m	Alligator weed	20	12	12	12
	Totals	542	251	226	226
		C. microsclerotia			
I	Privet	49	21	21	10
M	Privet	26	21	21	10
	Totals	75	42	42	20
		C. sasakii			
s	Bermuda grass	51	25	20	18
		C. sp.			
o	Fig	39	39	25	12
f	Fig	18	18	11	6
	Totals	57	57	36	18

FIG. 6 shows cultures of different strains grown under similar conditions.

Corticium microsclerotia. Only about one-half of these cultures were easily classified as belonging to this group (TABLE III). As many as 21 distinct cultural types were found among the 26 cultures isolated from one basidial mat. Some were similar to those observed among cultures of *C. solani.* The rate of growth varied considerably. The average increase in diameter ranged from a mere trace to 50 mm per 24-hour period (TABLE II). The color of the mycelium ranged from cream to deep brown. Sclerotial formation ranged from patches or tufts of sclerotial cells, about 0.5 mm across, to thick sclerotial bodies more than 10 mm across. In some cultures the large sclerotia were pocked where droplets of exudate had formed; in others they were covered with cottony mycelium. The topography of the cultures ranged from a felty appearance through varying degrees of tuftiness to a dense wooly growth (FIG. 8).

Corticium sasakii. Among the single basidiospore cultures from *Corticium sasakii* about one-fourth were atypical cultures (TABLE III). The rate of growth varied from an increase in diameter of 12.4 mm to 50.5 mm per 24-hour period (TABLE II). The color of the mycelium ranged from white to brown, but in most cases it was still light at the end of two weeks. Some of the cultures showed no sclerotial formation except dark patches on the surface of the agar which showed through when the plate was held to the light. Other cultures produced sclerotia of varying sizes and number. The most common form was an almost spherical, occasionally elongated, deep brown, pocked body averaging between 2 and 3 mm across. However, in one culture observed, the sclerotia had attained a diameter of over 10 mm (FIG. 7).

Corticium species. The single basidiospore cultures from the undetermined Corticium gave the greatest proportion of atypical cultures. Only about one-third were easily recognizable as belonging to the *Rhizoctonia* from which they came (TABLE III). On the average, the rate of growth was considerably less than that observed among the cultures in the other three groups. The

range in increase in diameter was from 2 to 39 mm per 24 hour period (TABLE II). The color of the mycelium ranged from cream to deep brown. Sclerotial formation ranged from a thin stroma of sclerotial cells through the flat, crusty, coalescing sclerotia typical of this group to separate, large, raised sclerotia, about 8 mm across. The flat, crusty sclerotia were usually located around the edges of the plates, whereas sclerotia of the other forms were either in rings or scattered over the surface of the agar. The mycelium in most cases was felty in apperance, but in a few cases it produced extensive aerial growth (FIG. 9).

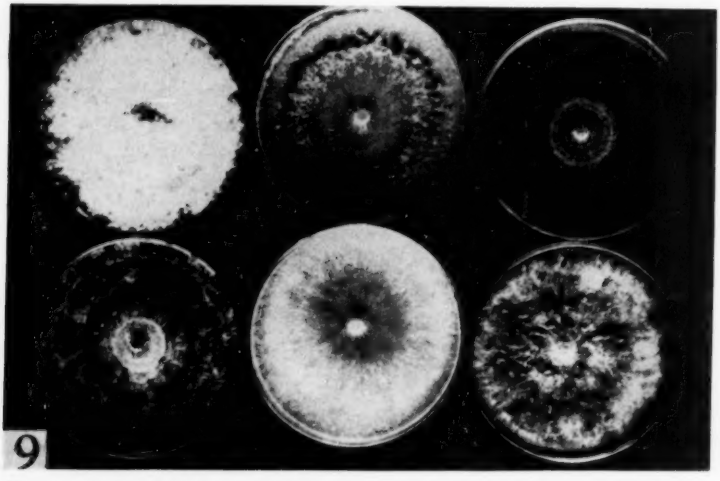


FIG. 9. Single basidiospore cultures of *Corticium* sp. showing variation in cultural characteristics among isolates from a single hymenium.

VARIATIONS IN PATHOGENICITY AMONG SINGLE BASIDIOSPORE CULTURES

The results of the pathogenicity tests (TABLE IV) indicated extensive variation in the ability of the single basidiospore cultures to damage the tissues of bush snap bean seedlings.

All four of the fungi produced lesions on the hypocotyl at the level of the soil and below. The only difference found in the lesions was in color. The cultures of *Corticium microsclerotia* and *C. sasakii* produced chocolate brown lesions, and the cultures

of *C. solani* and the undetermined *Corticium* produced reddish brown lesions. Among the cultures of *Corticium solani* pathogenicity ranged from slightly pathogenic to severely pathogenic. The cultures of *Corticium microsclerotia* showed a range of pathogenicity from non-pathogenic to slightly pathogenic. (Although

TABLE IV

PATHOGENICITY OF 113 SINGLE BASIDIOSPORE CULTURES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.) TO BUSH SNAP BEAN IN STERILIZED SOIL IN GREENHOUSE

Basidial mat	Number cultures with pathogenicity index of						Total cultures
	0	0.1–.5	0.6–1.0	1.1–1.5	1.6–2.0	2.1–2.5	
C. solani							
A	0	1	1	0	1	0	4
B	0	1	4	1	1	0	7
C	0	2	1	4	1	2	10
D	0	1	2	3	2	1	9
E	0	3	1	0	2	2	8
J	0	6	3	5	0	0	14
C. microsclerotia							
I	3	10	0	0	0	0	13
C. sasakii							
W	0	1	5	1	1	1	9
C. sp.							
O	3	11	3	7	5	0	29
F	7	11	2	0	0	0	20

0 = non-pathogenic.
3 = plants dead or dying.

these cultures were the least pathogenic of the four Rhizoctonias on the roots and hypocotyls of bush snap bean seedlings, in fields the aerial parts of older bush snap bean plants may be totally killed by web-blight.) The cultures of *Corticium sasakii* ranged in pathogenicity from slightly pathogenic to severely pathogenic. The cultures of the undetermined *Corticium* ranged in pathogenicity from non-pathogenic to moderately pathogenic.

In the damping off tests with ten cultures from a single mat of *Corticium solani,* the stands of cabbage, tomato, and eggplant varied extensively (TABLE V). An analysis of variance indicated significant differences among the cultures.

TABLE V

STANDS OF CABBAGE, TOMATO AND EGGPLANT IN SOIL INFESTED WITH 10 BASIDIOSPORE CULTURES OF CORTICIUM SOLANI BURT

Culture	Percent stand in relation to check		
	Cabbage	Tomato	Eggplant
B–5	42.8	82.5	51.3
B–6	8.5	30.6	37.5
B–8	56.5	51.9	62.5
B–10	32.7	47.2	33.6
B–11	1.6	11.9	3.8
B–12	1.6	44.4	37.5
B–14	4.6	83.3	14.1
B–19	80.8	99.2	104.2
B–21	63.4	85.3	71.2
B–23	2.3	40.8	1.1

F value = 810.64. F value exceeds 1% point.

DISCUSSION

When basidiospore cultures of the four fungi were compared a large number were found which could not be identified by cultural characters or pathogenicity on bush snap bean. This was found to be the case even when the spores were taken from small sections of basidial mats. Many of the atypical cultures produced by *Corticium microsclerotia* and the undetermined *Corticium* on fig would have to be considered as *C. solani* since they possessed no cultural characters which would enable them to be distinguished from that group. Cultures from basidiospores produced by *C. sasakii* and *C. microsclerotia* intergraded so that some of them could not be assigned to either group. (Since some of the cultures of *C. microsclerotia* produced sclerotia on agar as small as those on the host, it seems highly probable that the fungus which Matz described as *R. microsclerotia* (**9, 10**) was a strain possessing the same cultural characters).

The results obtained in these studies indicate that the sexual stages of these fungi were responsible for a wide variety of strains,

many of which differed from the type considered to be representative of each form. This suggests that the fusion nuclei in the basidia are heterozygous and that strains arise as a result of segregation. The possibility exists that several strains could have produced basidiomycetous stages at the same time in the case of each of the basidial mats from which basidiospores were obtained. However, the large number of strains found among the progeny from a single mat, for example, the 29 distinct strains among 75 cultures from a mat of *Corticium solani* and the 21 distinct strains found among the 26 cultures from a mat of *C. microsclerotia*, has led to the interpretation that segregation is responsible.

The relationship of these four fungi seems to be very close. The basidial mats are practically indistinguishable, and the basidiospores cannot be distinguished by their size. The basidia and sterigmata are similar except for slight differences in measurements, the ranges of which are overlapping.

It is the opinion of Rogers (**13**) that the differences between *Corticium microsclerotia* Weber and *Corticium solani* (Prill. & Del.) Bourd. & Galz. fall within the range of a single species—*Pellicularia filamentosa* (Pat.) Rogers. Since these two fungi represent the extremes in average measurements of basidia and sterigmata among the four Rhizoctonias used in these studies, the other two, *Corticium sasakii* Matsumoto and the undetermined *Corticium*, would be placed in the same species.

As has been pointed out in the first part of this paper, the diseases caused by the four Rhizoctonias are reasonably distinct, and when isolates are obtained from diseased plants, a rather clear-cut cultural strain is found associated with each disease. The form and size of sclerotia formed when the cultures were grown under definite conditions was found to be the most practical way of separating them in the laboratory.

Therefore, it is proposed that the species, which according to Rogers (**13**) is entirely parasitic, be sub-divided for reference to the causal organisms of the various diseases.

It was thought best to call the four Rhizoctonias used in these studies forms (formae specialies), because they are separated on the basis of cultural characters and diseases produced, and not on

morphological differences of the perfect stages, as are subspecies and varieties. This is in conformity with Article 14 of the International Code of Botanical Nomenclature of 1952 (3). The following descriptions have been prepared for their identification:

PELLICULARIA FILAMENTOSA (Pat.) Rogers, Farlowia **1**: 113. 1943.

1. Forma specialis **solani** (Kuhn) stat. nov.

Hypochnus solani Prill. & Del., Soc. Myc. Fr. Bull. **7**: 220, fig. *1991*. Muller, Biol. Reichsanstalt. Land- u. Forstwiss. Arb. **13**: 205–8, pl. *1, 2*. 1924.

Corticium vagum var. *solani* Burt ex Rolfs, Science N.S. **18**: 729. 1903. Missouri Bot. Gard. Ann. **13**: 295. 1926.

Corticium solani (Prill. & Del.) Bourd. & Galz., Soc. Myc. Fr. Bull. **27**: 248. 1911.

Corticium vagum sensu Burt, Mo. Bot. Gard. Ann. **5**: 128, fig. 3a. 1918.

Corticium vagum subsp. *solani* (Prill. & Del.) Bourd. & Galz., Hym. Fr. 242. 1928.

Botryobasidium solani (Prill. & Del.) Donk, Nederl. Myc. Ver. Med. **18–20**: 117. 1931; Rogers, Univ. Iowa St. Nat. Hist. **17**: 18. 1935.

The causal organism of basal stem and root rots of various plants.

Variable in the production of sclerotia in two weeks time on potato dextrose agar and Czapek's agar at 28° C. Some cultures produce numerous small (less than 2 mm), tawny to deep brown sclerotia which may coalesce to form flattened aggregations, and others produce only a layer of sclerotial cells over a large part of the agar or form a felty stroma.

2. Forma specialis **microsclerotia** (Matz) stat. nov.

Corticium microsclerotia Weber, Phytopath. **29**: 565, fig. *4, 7*. 1939. Mycologia **43**: 727. 1951.

The causal organism of web-blight of bean, fig, and various other plants. Numerous small, brown, superficial sclerotia, 0.2–0.5 mm in diameter on aerial parts of host.

Producing in two weeks time on potato dextrose agar or Czapek's agar at 28° C tawny to deep brown sclerotia, 3–7 mm in width, by 4–11 mm in length by 2–3 mm in depth.

3. Forma specialis **sasakii** (Shirai) stat. nov.

Hypochnus sasakii Shirai, Bot. Mag., Tokyo **20**: 319. 1906.
Corticium sasakii (Shirai) Matsumoto, Trans. Sapporo Nat. Hist. Soc. **8**: 115–120. 1934.

The causal organism of a leaf and sheath spot (banded sclerotial disease) on grasses and a leaf blight on various dicotyledonous plants.

Producing in two weeks time on potato dextrose agar or Czapek's agar at 28° C deep brown sclerotia of definite shape—almost spherical (flattened where they come in contact with the agar and occasionally elongated), varying in size from 1–6 mm in diameter.

4. Forma specialis **timsii** forma nov.

Parasitus luem in foliis *Fici Caricae* efficiens. Nulla sclerotia in hospite gignuntur. Diebus quattuordecim in agaro Solani tuberosi dextrosato 28° C numerosa sclerotia complanata et brunnea efficit, quae ita coalescunt ut collationes asperae (Sclerotiorum) per magnam partem superficiei agari formentur. Diebus quattuordecim in medio synthetico (e formula Czapekii) cui grammata quindecim agari addita sunt) sclerotia efficit globosa vel elongata, atro-brunnea infra sed tegmina colore dilutiore et gossypio similia gerentia, magnitudine 0.5–7 mm in diametro ac altitudine 0.5–4 mm.

The causal organism of a leaf blight on fig. No sclerotia produced on host.

Producing in two weeks time on potato dextrose agar at 28° C numerous flattened, brown sclerotia which coalesce to form rough, sclerotial aggregations on a large part of the surface of the agar and sides of the Petri dish. Producing in two weeks time on Czapek's agar at 28° C sclerotia which are round to elongate, deep brown underneath with a lighter colored, cottony covering, ranging in size from 0.5–7mm in diameter and 0.5–4 mm in depth.

SUMMARY

A study was made of the four Rhizoctonias known as *Corticium solani, Corticium microsclerotia, Corticium sasakii,* and an undetermined *Corticium* species from fig. Each causes a rather characteristic disease. Tissue cultures from diseased material gave rather distinct cultural types for each disease. However, when basidiospores were isolated, and the cultures obtained from them were compared, a large number of types differing in appearance, rate of growth and pathogenicity were found. In many cases strains from basidiospores of one Rhizoctonia could not be distinguished from strains obtained from basidiospores of the other three Rhizoctonias.

The four Rhizoctonias could not be distinguished by average measurements of basidiospores or basidia.

The taxonomy of these fungi is discussed and four forms belonging to the species *Pellicularia filamentosa* are suggested in place of giving them specific rank.

ACKNOWLEDGMENTS

The author wishes to thank Dr. St. John P. Chilton, under whose direction this work was done. Thanks are due also to Dr. C. W. Edgerton, Dr. T. C. Ryker, and Dr. E. C. Tims for their kind assistance.

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DEVELOPMENT OF THE CARPOPHORE OF BOLETINUS PALUSTER

ARTHUR C. GENTILE AND WALTER H. SNELL

(WITH 1 FIGURE)

The beautiful little *Boletinus paluster* (Pk.) Pk. is not common but when it does fruit, dozens to hundreds of carpophores are formed within a small compass. In 1941 Henry A. C. Jackson and the second author made an abundant collection near the railroad station at Vaucluse, Quebec, in a wooded area which deserves especial attention by those interested in the higher fungi because of the size of the woodland and the diversity of terrain and vegetation. This collection provided the material to add one more developmental study to the few made in the Boleti. The material was embedded, sectioned and stained by Miss Hilda Vilkomerson.

Space will be taken for no extended discussion because the general course of development of the carpophore can be ascertained from FIG. 1. When the primordia of the carpophores are 3–5 mm tall and the apical portion is 300–500 μ thick, the beginning of the pileus and the clothing layer are evident. FIG. 1, A, represents one of the larger conditions. When the pileus is only about 0.5 mm broad, it is well on its way to development, with a differentiated surface layer and a palisade layer of hyphal tips on the lower surface, and with the margin incurved and the marginal, hyphal weft approaching the stipe (FIG. 1, B).

When the pileus is 800 μ broad (FIG. 1, C), the hymenial palisade is more chromophilous, and at about 900 μ (FIG. 1, D) cystidia are evident, the marginal weft has made contact with the stipe, and the surface clothing appears much like that of the more mature stages.

The marginal weft may start to pull away from the stipe at the 1 mm stage (FIG. 1, F) or it may remain attached until the pileus is nearly 2 mm broad (FIG. 1, G), and even at the 4 mm stage

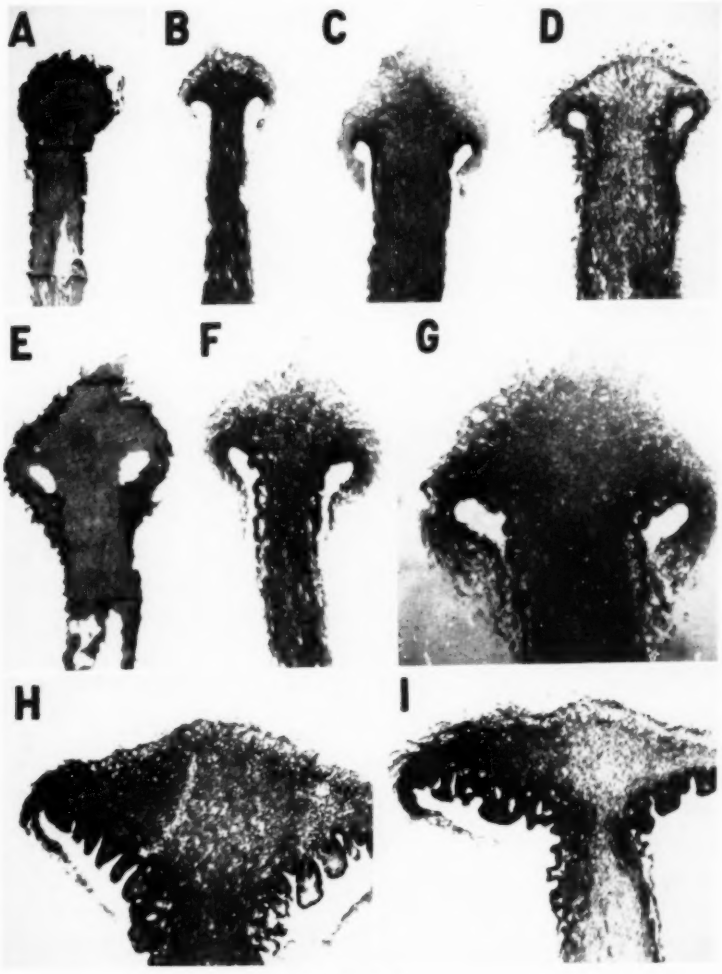

FIG. 1, A–I.

FIG. 1. Development of the carpophore of *Boletinus paluster*. A. Earliest stage, rather large, with knob-like pileus 550 μ broad, stipe slightly constricted at apex and surfaces of pileus and stipe covered with loose, projecting hyphae. B. Pileus 550 μ broad, showing definite pileus with incurved margin and marginal hyphae growing toward the stipe, palisade layer on the lower surface of pileus and a short way down the stipe, and surfaces of pileus and stipe of loose, hyphal tissue. C. Pileus 800 μ broad, with thick layer of

(FIG. 1, I) it has not retracted very far. In the mature carpophore, the veil remains appendiculate on the pileus and there is very little, if any, annulus on the stipe.

FIG. 1, G, H and I, show the irregular and lacunose development of a very boletinoid hymenophore.

The first mature basidia and basidiospores appear in small numbers at about the 1.5 to 2 mm stages of breadth of the pileus but are at all frequent only when the pileus is 2.5–3 mm broad, or between the stages represented in FIG. 1, H and I.

The type of development in *Boletinus paluster* is, therefore, pseudoangiocarpic.

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loose hyphae on the surface, more clearly defined palisade layer and further incurving of marginal hyphae. D. Pileus 880 μ broad, with pileic margin in contact with stipe completely enclosing the hymenial cavity, a definite hymenium of cystidia but no mature basidia, and looser clothing. E. Pileus 800 μ broad, showing complete and firm contact of the marginal tissue with the stipe, but details of all tissues lost because of poor focus. F. Pileus 1 mm broad, with marginal tissue slightly pulled away from the stipe and convoluted hymenium with abundant cystidia. G. Pileus expanded to 1.6 mm, with marginal tissues still in contact with the stipe and hymenophore lacunose as well as convoluted. H. Pileus 1.9 mm broad, almost completely expanded, with the marginal tissue free from the stipe but not far withdrawn, and tubes well started toward development. I. Pileus 4 mm broad and stipe about 1 mm thick with irregularity of tube-layer evident on pileus and upper stipe, and marginal tissue still much incurved. A–H about $\times 40$; I about $\times 20$.

CYTOLOGICAL OBSERVATIONS ON THE ZYGOTE OF MONOBLEPHARELLA[1]

FREDERICK K. SPARROW[2]

(WITH 1 FIGURE)

The Monoblepharidales, a small order of freshwater and terricolous phycomycetes, is unique in having a type of sexual reproduction involving a large non-flagellated egg and a disproportionately small, posteriorly, uniflagellate antherozoid. In the genus *Monoblepharis* Cornu (**1872**), species of which are typically found in cool, clear water, the egg completely engulfs the male gamete and the resultant zygote oozes out of the oogonium and becomes converted into a thick-walled oospore which remains attached to the orifice of the oogonium. In *Monoblepharella* Sparrow (**1940**), whose species have thus far been found only in tropical and subtropical soils, the flagellum of the male gamete persists after fertilization, the zygote emerges and swims away propelled by that flagellum. Such a unique type of behavior has been verified by Shanor (**1942**) and Springer (**1945**) in other species of the genus and has also been recently discovered by Johns (**1952**) in species of the well known genus *Gonapodya*.

In such a type of sexual reproduction as found in *Monoblepharella* one of the points of greatest interest is to determine just what nucleus presides over the activities of the flagellum during the motility of the zygote. Is there immediate nuclear fusion and does the diploid nucleus function in this respect, or is it one or other of the gamete nuclei?

Owing to the small size of the structures involved, sectioning of material did not seem feasible. Rather, methods similar to those employed by Kniep (**1929**) and others in studying the nu-

[1] Contribution No. 979, Botany Department, University of Michigan.

[2] Acknowledgment is made to the Executive Board of the Horace H. Rackham School of Graduate Studies for financial support given this project.

clear condition in the motile structures of *Allomyces* were employed. Cover slips from hanging drop cultures in which zygotes were swimming were removed from the Van Tieghen rings and the material killed, fixed, and strained on the cover slip.[3]

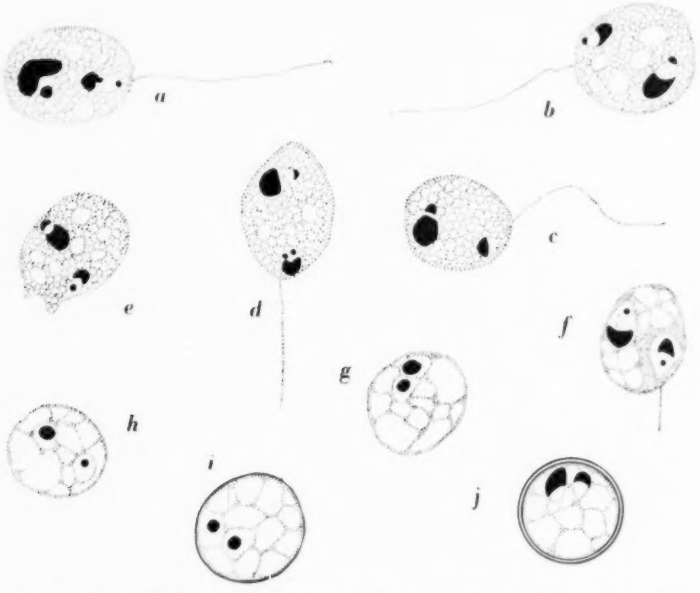

FIG. 1. *Monoblepharella taylori*. All Figs. × 1300. a–d, f. Killed, fixed and stained zygotes bearing flagella. In each instance the smaller nuclear apparatus of the male, with its nuclear cap lies near the flagellum, the larger apparatus of the female gamete more anterior. In a, f, the male nuclear apparatus and flagellum are in the position they probably assume during zygote motility. e. Zygote devoid of flagellum, beginning to assume amoeboid movement before rounding off and encysting. g, h. Rounded off zygotes prior to wall formation. i. Beginning of wall formation. j. Nearly mature oospore.

Owing to the small size of the nuclear apparatus (4.75 μ female gamete, 2.5 μ male gamete, including "nuclear cap") and the limitations inherent in the use of whole mounts, no attempts were

[3] Of the various standard killing and fixing fluids, Bouin's seemed to be most serviceable. Iron-alum hematoxylin stain, destained with picric acid in 70% alcohol gave a particularly sharp differentiation of the nuclear apparatus.

made to study the finer details of the nucleus itself. Indeed, for purposes of the study as outlined, this was not necessary. It will be recalled that a highly characteristic feature of the nucleus of all posteriorly uniflagellate motile bodies formed by Phycomycetes is the presence on the anterior periphery of a deep-staining "nuclear cap" of unknown function. This is ordinarily not regarded as being a part of the nucleus proper. At the opposite side is a deep-staining granule, the nucleole, intimately connected with the flagellum. Since nuclear cap size always bears a definite and constant relationship to the size of the motile cell which contains it, and since the sperm and egg are considerably different in size (sperm $5\mu \times 3\mu$ compared with $13\mu \times 10\mu$), attention may be directed primarily to the nuclear caps.

In FIG. 1, a–d, f, are shown stained zygotes with their flagella. Within the zygote are seen two nuclei with distinctly different sized nuclear caps. In each instance the larger of the two nuclear structures is near the anterior portion of the zygote and the smaller near the point of insertion of the flagellum. In FIG. 1, e is shown a zygote which has evidently lost its flagellum and is about to move amoeboidly before encysting. Here, too, the nuclear structures are distinct and different sized. It is evident, therefore, from these few examples of many similar ones which might be shown, that the nuclei of the two gametes remain distinct within the motile zygote and that the smaller, undoubtedly male gamete nucleus maintains its connection with the flagellum and governs its activities.

After the zygote comes to rest in the water and loses its flagellum, it rounds up and there is gradually secreted a somewhat thickened wall, which at maturity of the oospore is smooth and somewhat brownish. FIG. 1, g, h, indicate that during this encystment and oospore wall formation the nuclear caps, and probably the nuclei, remain distinct. In FIG. 1, j, a recently matured oospore is shown and here, too, the nuclear caps remain distinct.

From this brief study it is apparent that the nucleus of the male gamete rather than a fusion nucleus presides over the activities of its flagellum during motility of the zygote and that the nuclear caps, and probably also the nuclei, remain distinct in the early

stages, at least, of oospore formation and maturation. Fully mature oospores are difficult to stain *in toto*. From what can be determined, however, only a single nucleus is present. Nuclear behavior in the oospore appears to parallel that found in *Monoblepharis* by Lagerheim (**1900**) and Laibach (**1926**) where here, too, the nuceli lie side by side until an advanced stage of wall maturation, when they eventually fuse.

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THE GENUS BEAUVERIA, MORPHOLOGICAL AND TAXONOMICAL STUDIES OF SEVERAL SPECIES AND OF TWO STRAINS ISOLATED FROM WHARF-PILING BORERS

RHODA W. BENHAM[1] AND JOSE L. MIRANDA[2]

(WITH 5 FIGURES)

The genus *Beauveria* is of interest both historically and from the fact that it contains many well known insect parasites, including the agent of "*la muscardine*" or silkworm disease. This is one of the most commonly known species, because of its economic importance and because of the epoch-making studies of Pasteur. It has acquired recognition among bacteriologists and medical mycologists as it is usually cited in text books on these subjects as the first instance in which a disease in man or animal was proven to be due to a definite micro-organism. It was as early as 1835 that Bassi studied and described the disease and thus established precedents for future workers. To quote Gay, 1935, "The proven pioneer work of Bassi on the muscardine disease of silkworm to which we have already referred, established a standard for proving the etiological relationships of a micro-organism found in diseased tissues. He realized the specific significance of the particular fungus involved and transmitted the malady from diseased to healthy worms."

Bassi was not a mycologist and therefore sent the cultures to Balsamo-Crivelli for study, who placed the fungus in the genus

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[2] Squibb Fellow; on leave from the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

Botrytis, naming it *Botrytis Bassiana* (Balsamo **1835**). Later studies by other authors indicated that the organism was similar to other insect parasites being isolated from various localities and that the characteristics were not those of a *Botrytis.* However, other species were described under this name. Delacroix (**1891**) described as *Botrytis tenella* a similar species obtained from cockchafer. Giard (**1891**) showed that this fungus had been described by Link in 1809 as *Sporotrichum densum.* Giard, however, noted that the fungus formed coremia and adopted the name *Isaria densa* (Link) Fr. Delacroix did not agree with Giard and retained the name *Botrytis tenella.*

Saccardo (**1886**) described a *Botrytis tenella* as a sub-species of *B. Bassiana* in Sylloge Fungorum **4**, p. 119. He did not, however, mention the characteristic conidial heads of the *Botrytis Bassiana* group and there seems to be some doubt as to whether the species is allied to *B. Bassiana.* Spegazzini (**1880**), in South America, described from dead carabid beetles another species as *Sporotrichum globuliferum.* This name has been applied, as pointed out by Dodge, in America to the fungus which parasitizes the chinch bug, *Blissus leucopterus.* Pettit (**1895**) studies this species and observed the purplish color mentioned below. Thus it will be seen that two generic names have been given to what appear to be similar insect parasites, *Botrytis* being the name more commonly used by European mycologists, and *Sporotrichum* that preferred by the Americans. The organisms in question do not fit in either group. Vuillemin (**1912**), realizing this, created the new genus *Beauveria* for these forms, and characterized the genus by the unique type of conidiophore, suggesting a cyme or sympodium. The sporogenous filament forms a prolongation of the phialide and the disposition of the spores gives a zigzag arrangement. *Botrytis Bassiana* then became *Beauveria Bassiana* (Balsamo) Vuill. Picard (**1914**) transferred Spegazzini's species to *Beauveria* as *Beauveria globulifera* (Speg.) Picard. Beauverie (**1914**) added a new species, *Beauveria effusa,* and published a critical study of the genus. He concludes that there are four species, which he lists as follows:

(1) *Beauveria Bassiana* (Balsamo) Vuillemin 1911
(2) *Beauveria effusa* (Beauverie) Vuillemin 1911
(3) *Beauveria densa* (Link) Picard 1914
(4) *Beauveria globulifera* (Spegazzini) Picard 1914

Beauverie states that these species are all very similar, the most startling differences being in the gross characteristics. They are all known to be insect parasites.

Petch (**1924**) also gives a critical survey of the group and reports on studies with strains from various sources. He concludes that the morphological differences between alleged species are slight and that from recorded experiments it does not seem probable that the parasitism of a given species is restricted to one species of insect or even one group of insects. Lefebvre (**1921**) had shown that both *B. Bassiana* and *B. globulifera* could attack the corn-borer *Pyrausta nubilatis*. The strain *B. Bassiana* appeared more infectious, however. He gives illustrations that are typical of the genus and agrees with others that the two forms are similar morphologically but that *B. Bassiana* produces a chalky growth and *B. globulifera* a flocculent growth.

In spite of the vast literature on these insect-parasites and the many excellent illustrations of their characteristic morphology we find a report by Limber (**1940**) on "A new form genus of the Moniliaceae." He found his fungus in dead roots of a *Yucca* plant of Cuban origin. The conidium-bearing filaments had a zigzag appearance and since it seemed similar to the rachis of a wheat spike, he created a new genus and named it *Tritirachium* and the type species *dependens*. This, he states, can readily be distinguished from other members of the Moniliaceae by the zigzag form of the fertile portion of the conidiophore. The only other reference he was able to find in the literature to this zigzag formation was that of Ferraris (**1912**), who described under the name *Sporotrichum flavicans*, a fungus which was found on the underside of cork placed in a flask of picric acid. Limber describes a second species under the name *Tritirachium album*. This he received from Dr. Charles Thom, who found it associated with a colony of *Penicillium intricatum* in material he had obtained from Dr. Oscar Richards, who had recovered it from a book in the basement of

the Marine Laboratory in Woods Hole. Limber's illustrations differ from the typical *Beauveria* picture in some respects but the zigzag arrangement of the spores is very evident. However, he makes no mention of the genus *Beauveria*.

Finally the whole subject was brought to the field of the medical mycologist, which justifies our interest in these forms, when Langeron (**1934**) reported on his study of a fungus isolated from a case of infection of the eye in a 17 year old girl from Cairo, Egypt (case of Dr. Elia Lichaa). Langeron stated that the fungus had all the characteristics of the genus *Beauveria*. He gave a careful description such as to leave no doubt that he was indeed dealing with a *Beauveria*, but he stated that it differs in some respects from the described species. For one thing it grows at 37° C, whereas the others will not. Langeron considered it a new species and gave it the name *B. Brumpti*. However, upon the publication of Limber's paper Langeron noted the similarity of the illustrations of *Tritirachium dependens* to his *Beauveria Brumpti* and transferred his organism to the new genus, along with all of the other strains of *Beauveria* reported as human pathogens. There seems little doubt that *Tritirachium* differs from *Beauveria* in having a more constant verticillate arrangement of the sporophores, which are long and slender, not swollen, but with a thick prominent wall, distinguishing the conidiophore from the other hyphae, much as in certain *Aspergillus* species.

To further complicate the picture, Langeron states that Saito (**1939**), in Japan, had encountered this type of condiophore and created for it the genus *Spirotrichum* with the type species *S. purpureum*. Van Beyma (**1942**), in studying a similar organism, eliminated the genus *Spirotrichum* on the basis that Saito had not given a Latin description and adopted the genus *Tritirachium* Limber (**1940**), naming his culture *Tritirachium roseum* Van Beyma. Van Beyma added three species to the genus, Saito's and two of his own, making a total of eleven.

The fungi which form the basis of this study were isolated from the larvae of the beetle *Naecrda melanura* by Dr. B. O. Dodge. The larvae were obtained from wood from piles at Whitehall Street Ferry Terminal, New York City. In a recent publica-

tion, Dodge (**1951**) reported his findings, stating that in addition to several common saphrophytes, such as *Mucor, Aspergillus, Trichoderma* etc., he had obtained two strains of fungi which were parasitizing the larvae, one chromogenic, the other non-chromogenic. He placed them both in the genus *Beauveria.* This is believed to be the first report of such parasites from the beetle *Nacerda melanura.* Further studies were indicated for a more precise species identification and a clearer understanding of the host-parasite relationship between these piling borers and the fungus pathogen.

MATERIALS AND METHODS

The two strains isolated by Dodge (**1951**) designated as #1 chromogenic and #2 non-chromogenic were studied both as mass isolated strains and as monospore strains. For comparison of morphology a strain of *Spicaria* isolated from the same source was examined. In addition representative strains of each of the four species mentioned above and one of *Tritirachium dependens* were studied. The sources of these strains and the label on each when received is given below.

1. *Beauveria Bassiana* A. T. C., *Beauveria Bassiana* (Bals.) Vuill. #*9835*, J. H. Stevenson, U.S.D.A. W. D. Wylie
2. *Beauveria densa* A. T. C., *Beauveria densa* (Link) Vuill. #*9432*, Johanna Westerdijk C. B. S.
3. *Beauveria densa* C. B. S., *Beauveria densa* (Link) Vuill. fr. Halticid *760*, 51134
4. *Beauveria effusa* A. T. C., *Beauveria effusa* (Beauv.) #*9453*, Johanna Westerdijk C. B. S.
5. *Beauveria globulifera* C. B. S., *Bauveria globulifera* (Speg.) Picard *763*, 51137
6. *Tritirachium dependens* Strain PQMD 9–2, isolated at Harvard University, 1944, from side leather of shoe from Bougainville Island. Identification by W. L. White

We are indebted to Miss Julia Coffey of the Division of Laboratories and Research, New York State Health Department, for these cultures.

The cultures were studied on a variety of media for gross characteristics and in hanging drop and slide cultures. The slides were examined at weekly intervals for 4 weeks. Teased preparations from plate cultures were also examined. The following media were used in these studies:

Sabouraud's dextrose agar (Difco)

Potato agar with and without dextrose

1. Pared-potato water 300 gm potato 500 ml distilled water	Boil potatoes in water until mealy, filter through cheesecloth, make up to volume; for dextrose agar add 20 gr dextrose.
2. Agar water 20 gm agar 500 ml distilled water	Heat to dissolve; add to (1), tube and sterilize 25 minutes at 15 lbs pressure; pH as made up = 5-6.

Potato plugs

Casein hydrolysate medium (C-50)

$MgSO_4 \cdot 7H_2O$	0.18	grams per liter
Dextrose, C. P.	40	grams per liter
Sorensen's N/15 phosphate buffer mixture to give a final pH of 7.0	100	ml per liter
"Purified" agar	20	grams per liter
Casein hydrolysate "vitamin free"	0.5	percent
Distilled water to make	800	ml

Czapek agar (Dox and Thom's modification)

Agar	20.0	gm
Sucrose C. P.	30.0	gm
$NaNO_3$	2.0	gm
$MgSO_4$ crystals	0.5	gm
KCl	0.5	gm
$FeSO_4$ crystals	.01	gm
K_2HPO_4	1.0	gm
Distilled water	1000.	ml

Add agar to 900 ml water (hot), melt in autoclave (10 minutes 15 lbs pressure); add other ingredients one at a time in order mentioned, dissolving each chemical in turn, before adding next one. Dissolve K_2HPO_4 in 100 ml water (cold) and add slowly to agar mixture, with vigorous stirring. Distribute in small flasks. Sterilize by autoclaving at 15 lbs pressure for 15 minutes. No pH adjustment necessary. Acid reaction.

Corn meal agar

Corn meal 62.5 gm
Water 1500 ml

Heat at 60° C for 1 hour, filter through paper and make up to 1500 ml, add 19 gm agar, heat in Arnold sterilizer for 1¼ hours, filter through cotton and tube. Sterilize in the autoclave for 25 minutes at 15 lbs pressure. pH as made up is about 6.8 to 7.

Wort agar (Difco)

Sections through the mummified larvae were likewise studied both from those where infection occurred spontaneously and from one in which the infection was produced experimentally by Dodge, who sprayed an apparently healthy larva with a suspension of spores.

EXPERIMENTAL RESULTS: PARASITIC STAGE

The infected larvae appear sick and die in a short time, and almost immediately become covered with a dense coating of a white mold. This white covering, quoting Petch (**1924**), is what gave the name *"calcino,"* meaning chalky, to the silkworm disease in Italy and *"la muscardine"* to the same disease in France, the latter from the supposed resemblance to a sugared almond. Several transfers from this white coating yielded what appeared to be pure cultures of the same fungus. Sections through the mummified larva showed the entire larva to be filled with a mass of branching hyphae (FIG. 1 A, B). The tissues of the larva have been practically replaced by the fungus. These hyphae were much thicker than those on the surface of the larva, about twice the diameter. On the outer edge of the external mycelial mass one notes the formation of the spherical clusters or glomerules of spores that are characteristic of this fungus (FIG. 1 D, E).

Balsamo (**1835**), who made careful studies of Bassi's culture, was successful in artificially infecting silkworm larvae. He noted that the infected worms took on a red color, and when this red material was teased out from the larvae and examined, it was found to consist of mycelial threads and spores. By planting fragments of this material he obtained the fungus which he named

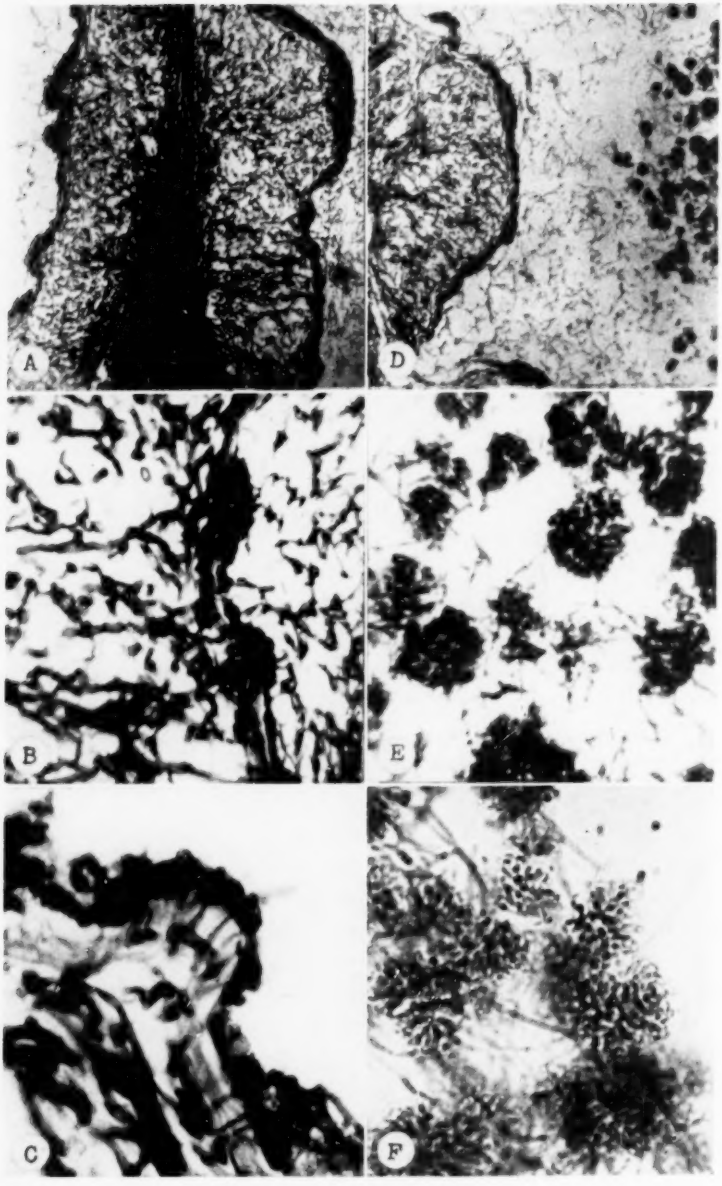

FIG. 1.

Botrytis Bassiana. Balsamo noted that the fragments of threads which he teased out from inside the dead insect were thicker than the *Botrytis* filaments. This agrees with our finding. The presence of the red pigment is significant, since later authors distinguish between a red and white muscardine. *Beauveria Bassiana* is given as the cause of the white muscardine, and other species, such as *effusa* and *densa*, of the red. As our study progressed, we were forced to conclude that the earlier workers placed more emphasis on pigment production than is warranted, since it is such a variable factor.

It is believed that these larvae are infected through the integument rather than through the intestinal tract. Picard states this, claiming that the infection is rapid, the worm dies and on the mummy are produced the external hyphae and spores which form the chalky white coating so characteristic of the disease. Extension of threads from the inside to the outer surface were noted in one of our sections (FIG. 1 C). Further studies of the parasitic stage of this group of fungi is indicated in order that we may better understand and distinguish the various species of this interesting genus.

CULTURAL STUDIES: GROSS CHARACTERISTICS

The strain which we designated as Dodge's #1 chromogenic formed at first a flocculent, cottony growth in Sabouraud's dextrose which soon became covered with a powdery mass, whereas the strain designated as Dodge's #2 non-chromogenic developed a flat, downy to felt-like white growth. They both showed a slight orange pigment on the reverse of the colony. On Potato plugs strain #1 formed a similar flocculent growth and turned the potato an intense purple red, while strain #2 produced a flat

FIG. 1. Sections through infected larva. A. Portion of longitudinal section showing the entire tissue of the larva replaced by fungus filaments, × 90. B. Outer edge of worm at higher magnification with coarse filaments within, finer ones on outside, × 450. C. Enlarged view of a portion of outer edge showing hyphae passing through integument, × 850. D. Clusters of spores which have formed on the fine external hyphae, × 90. E. High power view of clusters, × 450. F. Similar clusters from culture, × 650.

downy growth, somewhat wrinkled, but with no pigment. Monospore strains of these two isolates gave the same characteristics as the original culture. On potato plugs the chromogenic strain developed finger-like processes, so-called "clubs," or coremia (FIG. 5 A). These were never seen in the non-chromogenic strain.

On potato dextrose agar a marked difference again is noted between the two cultures; strain #1 forms in concentric rings a flat chalky powdery or granular growth, which cracks as the culture

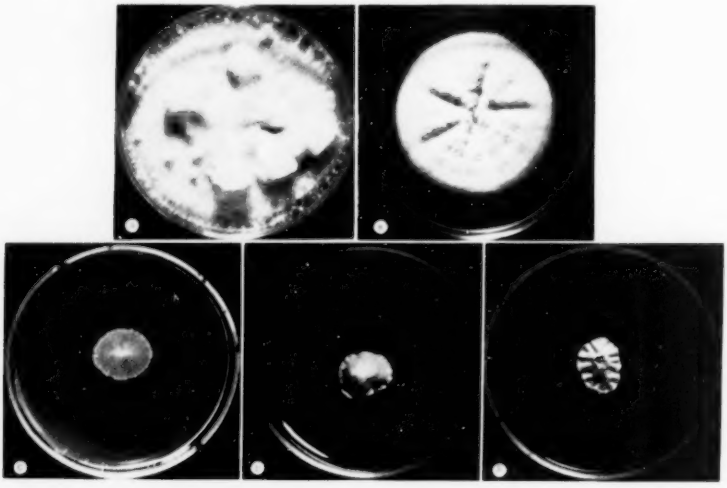

FIG. 2 A, B, E. Three-weeks-old Sabouraud's dextrose cultures of Dodge No. 1 chromogenic strain. A. Dodge No. 2 non-chromogenic strain. B, and *Tritirachium dependens*. E. C, D. Response of *B. densa* strain No. *1283* to thiamine. Cultures two weeks old on 0.5% casein medium, C, and on 0.5% casein medium plus 0.1% thiamine hydrochloride, D.

ages. A slight pink-rose pigment is formed both on the surface and on the reverse of the colony (FIG. 3 E). Strain #2 produces downy to fluffy white aerial mycelium (FIG. 3 D). The reverse shows a slight cream-yellow to rose tinge. On potato agar minus dextrose the growth is similar to texture in both instances but not so abundant (FIG. 3 A, B).

On the casein hydrolysate medium the growth of the two strains was not so easily differentiated, as both strains initially formed

a white downy to cottony growth. Later on, however, strain #1 developed the powery masses (FIG. 3 J). The pigment on the reverse of the colony of the chromogenic strain was a deep rose or purple whereas the non-chromogenic strain formed a rose buff pigment.

On Wort the growth is similar for each, with strain #1 showing a stronger pigment on the reverse of the colony.

The flat, powdery, or chalky growth is noted for the chromogenic strain on Czapek's medium with the reverse of the colony a dull rose brown, whereas the non-chromogenic race gave a more fluffy growth and a yellow pigment on the reverse.

The growth was much reduced in both instances on corn meal agar. With strain #1 the growth is partly submerged and transparent with scattered zones of powdery, aerial mycelium, indicative of a massive spore formation. A slight rose pigment on the reverse was noted when the strain was first isolated but later was lost. Strain #2 also shows a partly submerged growth and no pigmentation on the reverse.

MICROSCOPIC MORPHOLOGY

Both of our strains produce a fine, branching, septate mycelium with flask-shaped sporophores or "phialides." The conidiophores may be borne along the hyphae singly, in pairs opposite each other, or in groups. They often occur in whorls or verticils. The phialide becomes prolonged into a filament upon which the spores are borne basifugally. A spore is borne on the axis of the sporophore attached to a tiny sterigma which terminates the axis at that point. The sporophore then grows in the opposite direction producing a second spore, then in like manner a third and fourth until a zigzag arrangement is obtained with the youngest spore at the tip. The older spores may become dislodged but the sterigmata remain attached to the axis (FIG. 4 C). With the chromogenic strain it is usual to see two spores remaining attached at the tip (FIG. 4 F) whereas with the non-chromogenic there is usually only one (FIG. 4 G). In the chromogenic strain the conidiophores occur more often in whorls or verticils and the spores are numerous, many remaining attached for some time

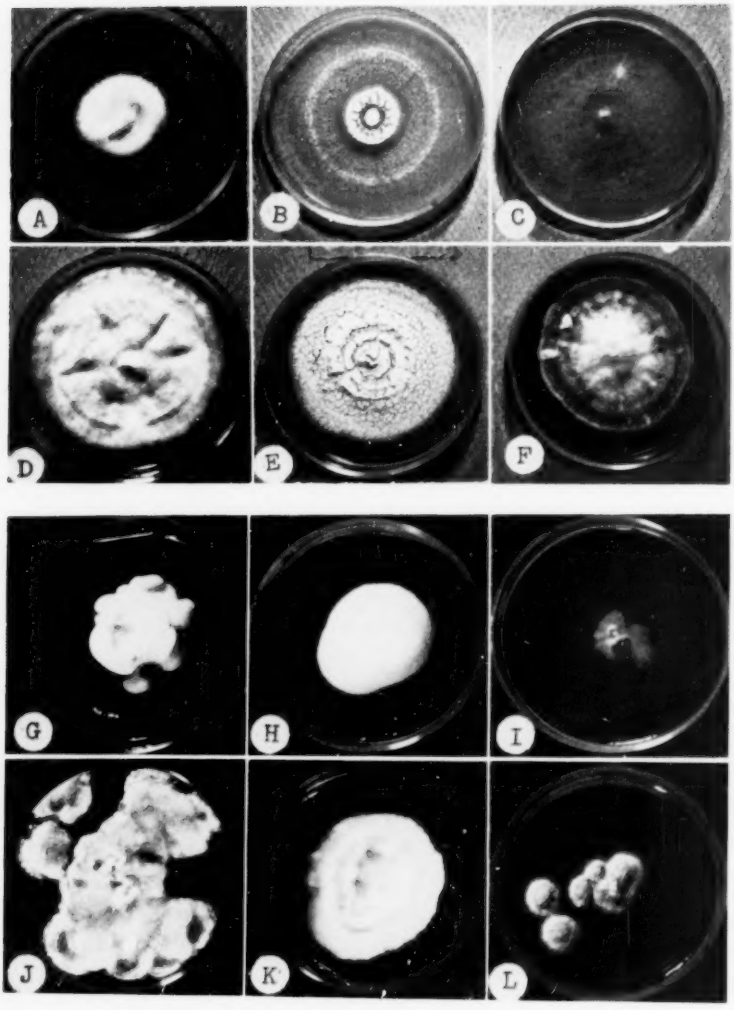

FIG. 3. Upper half. Three-weeks-old cultures of Dodge No. 2 non-chromogenic strain, Dodge No. 1 chromogenic strain, and *Spicaria* on potato agar, A, B, C, and the same on potato dextrose agar, D, E, F. Lower half. Three-weeks-old cultures of six species of *Beauveria* on casein hydrolysate medium; G. *B. Bassiana*, H. *B. effusa*, I. *B. densa*, J. Dodge No. 1 chromogenic, K. Dodge No. 2 non-chromogenic, and L. *B. globulifera*.

so that dense spherical clusters or glomerules are formed. The masses of spores give the pink powdery appearance to the cultures. Similar spherules occur on the surface of the larvae (compare FIG. 1 E, F). This powdery appearance is not found in strain #2, as there are no large spherical clusters or spores formed. Numerous spores are produced, however, but on scattered conidiophores. The branched hyphae bear many flask-shaped sporophores along the sides, often opposite each other. The swollen base of the sporophore is drawn out and usually one spore remains attached. The drawn-out filament has a curved appearance but a pronounced zigzag is difficult to see (FIG. 4, D, G). Variation in the sporophore are common in each strain.

Size and shape of spores has been used to differentiate species of *Beauveria*.

The spore measurements for our two strains are within the limits of species variation. They tend to be ovoid, though in both strains some spherical spores may be found. In strain #1 spores range from 1–2.3 × 2.3–3.3 μ, and in strain #2 spores from 1–2 × 1.5–3 μ are to be found. These measurements were made from corn meal and Czapek agar by means of the camera lucida and oil-immersion lens and did not seem to vary much with the medium. Because of the type of arrangement of the sporophores or conidiophores which predominates it is easy to distinguish between our two strains microscopically (FIG. 4 D, H).

The species of *Spicara* studied was easily identified by the chains of spores borne on phialides and by the gross appearance of the culture, which was a grayish brown color and fluffy in character (FIG. 3 C, F).

OTHER BEAUVERIA SPECIES

Of the other *Beauveria* species listed above all were shown to have the characteristic sporophore (FIG. 4 B, I, J, K).

It would be very difficult to distinguish the strains of *B. Bassiana* and *B. effusa* studied from each other or from our chromogenic strain, either from their gross characteristics or the morphological structure. The two strains labelled *B. densa* are similar to one another, but differ from the other strains in that they have

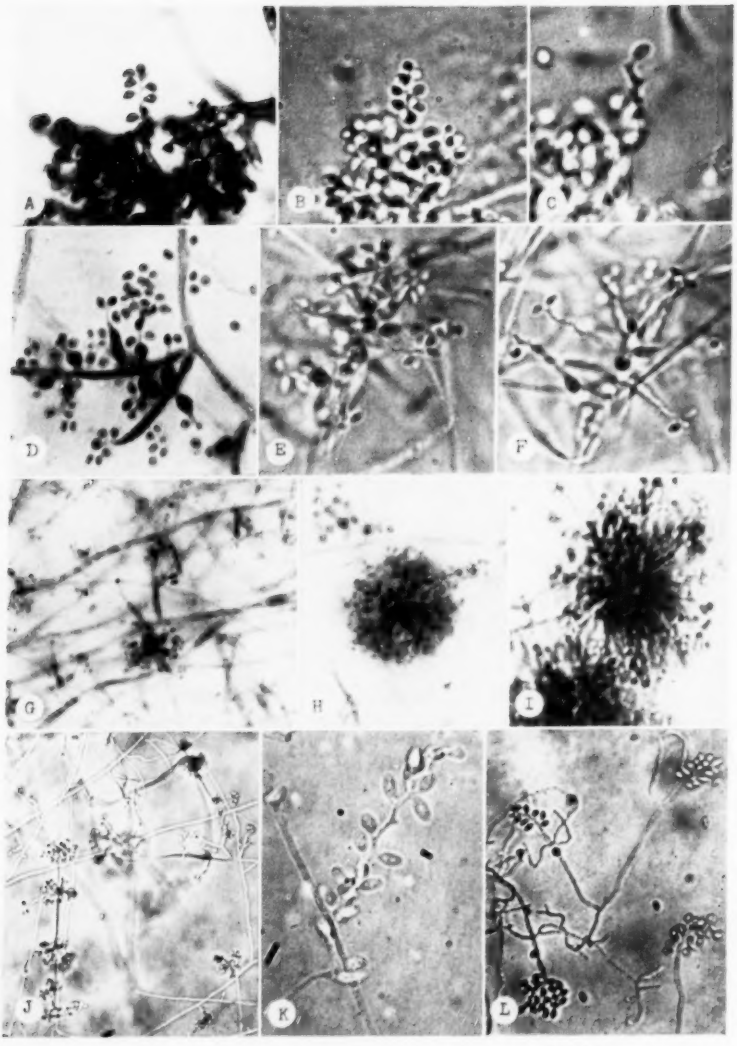

FIG. 4. Photomicrographs from slide cultures. A, B. Edge of spore clusters showing zigzag arrangement of spores: A, in section from infected larva; B, from slide culture of *B. Bassiana*, × 1450. C. Conidiophore of Dodge No. 1 chromogenic strain showing sterigmata to which spores were attached, × 2000. D, G. Characteristic conidiophores of Dodge No. 2 non-chromogenic strain. G, a young cluster, × 400. D, × 1450. E, F. Dodge

somewhat larger and more distinctly elongated spores. The conidiophores are not swollen, but long and slender (FIG. 4 K). Another interesting point which serves to distinguish these two organisms from the others under discussion is the fact that they appear to have a vitamin deficiency. It was noted that on the casein hydrolysate and the Czapek media, neither of which contain vitamins, the *densa* strains failed to grow or produced only a slight growth (FIG. 3 I). When thiamine hydrochloride was pipetted over the surface of these plates, or added to the medium, growth increased and soon equalled that of the other strains. This would seem to indicate that the *densa* strains require thiamine for optimum growth (FIG. 2 C, D).

B. globulifera showed a similar though less pronounced deficiency for this vitamin (FIG. 3 L). This strain differed in its flat, downy, more compact growth, in the yellow color of the aerial mycelium, and in the deep orange diffusible pigment which formed on the undersurface of the colony. It colored the potato a greenish-yellow rather than red; this has been reported by others for this species. *B. globulifera* according to earlier workers resembled in its microscopic structure both *B. bassiana* and *B. effusa*. Picard (**1914**) illustrated under the name *B. globulifera* the glomerules found to be so characteristic for our chromogenic strain and the one which we received as *B. bassiana*. The dense, closely-matted growth of the strain of *B. globulifera* studied obscured the individual spherules, but that they were formed is well illustrated in FIG. 4 J. Variation between the flat, more compact type of growth and the flocculent or cottony type may be noted in a given strain. This was well demonstrated by our strain of *B. bassiana*. A Sabouraud-dextrose plate culture developed in two sectors, one flat, the other more flocculent. Transfers were

No. 1 chromogenic strain, two optical levels of a spherical cluster, lower level showing swollen bases of phialides, E, and F showing the terminal zigzag, × 1450. H. Same strain showing fully formed spherical clusters, × 400. I. Clusters of *B. Bassiana;* note zigzag conidiophore similar to F, × 730. J. Young clusters of *B. globulifera*, × 300. K. conidiophore of *B. densa*, showing more oval spores and no swollen base, × 1450. L. Conidiophore of *Tritirachium dependens* showing similarity to *Beauveria*, × 650.

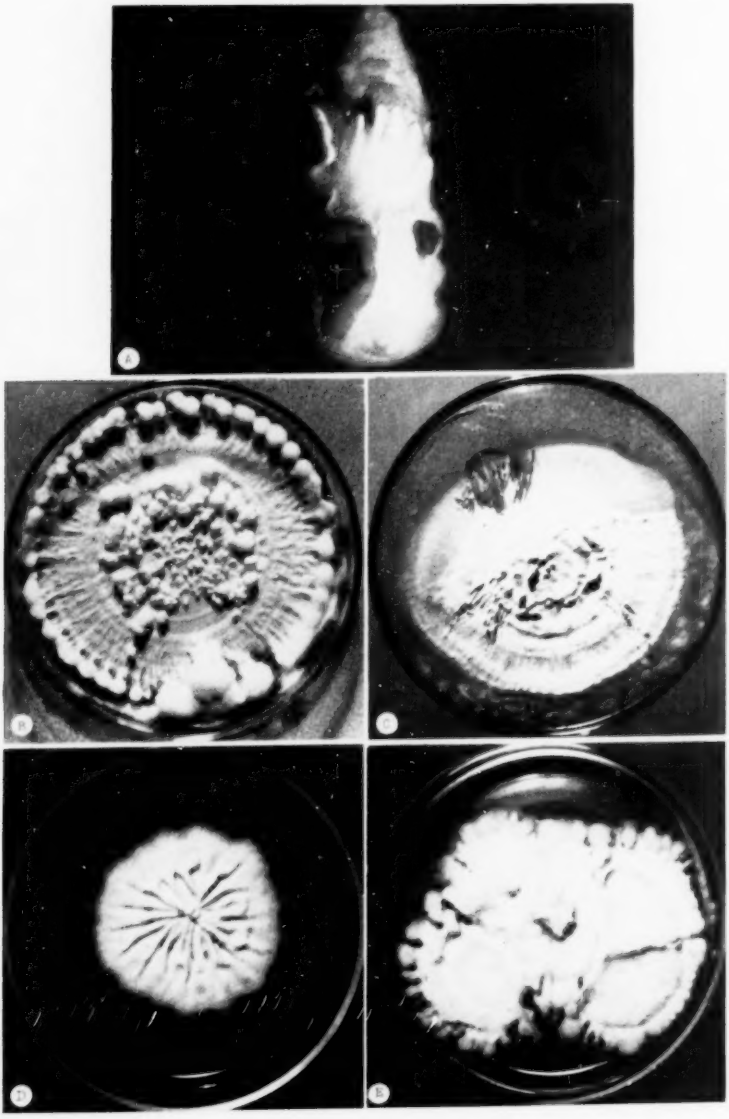

FIG. 5. Cultures of Dodge No. 1 chromogenic strain and *B. Bassiana*. A. Potato agar plug culture of Dodge No. 1 chromogenic showing coremia and dark patches of pigment. B. Three-weeks-old culture of *B. Bassiana*

made from each sector to wort agar plates with the following results: the flat sector resulted in a folded, downy colony and the more flocculent sector developed a fluffy, cottony growth with characteristic coremium formation (FIG. 5 B, C, D, E). These characteristics were maintained through repeated transfers. Monospore strains of these two types should be obtained and observed for further variation; nevertheless, this observation serves to point out the variability of the gross appearance of this group of micro-organisms.

TABLE I

COMPARISON OF STRAINS OF BEAUVERIA STUDIED

Strain	Predominant type of growth on Sab. D	Coremia	Pigment	Spore measurements	Predominant types of conidiophore
B. Bassiana	Flocculent or wool-chalky, cream	+	Slight	1–1.7×1.2–2.5μ	Spherical clusters
B. effusa	Downy-cottony, white	+	Not noted	1.2–2×1.3–3μ	Spherical clusters
Dodge Chromogenic	Flocculent or woody, chalky	+	+	1.2–3×2.3–3.3μ	Spherical clusters
Dodge Non-Chromogenic	Flat, compact, downy-white	–	–	1–2×1.5–3μ	Scattered phialides, few in verticils
B. densa (1)	Downy-white	+	Not noted	1–3×2.6–4.3μ	Phialides reduced
B. densa (2)	Downy-white	+ Slide culture	Not noted	1.5–2×2–4.8μ	Phialides reduced
B. globulifera	Compact, felt-like, firm, cream to yellow	Not noted	Yellow to greenish to orange	1–2.1×1.5–3.3μ	Compact mass of phialides

Tritirachium dependens is distinct both in gross and microscopic appearance. On Sabouraud's agar a compact, felt-like, convoluted tan colony forms with a deep purplish brown color on the undersurface (FIG. 2 E). The conidiophore, as noted above, is long and slender with spores borne in a zigzag arrangement (FIG. 4 L). The swollen phialide-like structure is lacking.

on wort agar showing coremium formation. C. *B. Bassiana* on Sabouraud's dextrose agar showing sectors. D, E. Transfers from sectors to wort agar, D from upper, flatter sector, E from lower, more fluffy sector. Note similarity of E to B, the parent culture.

The characteristics of the above-named species and our new isolates are summarized in TABLE I.

DISCUSSION

It will be noted from the above table that our chromogenic strain is very close to *B. Bassiana* and *B. effusa*. Beauverie long ago noted the close relationship of these two species. He differentiated them primarily upon the gross characteristics, a flocculent versus a chalky growth, and red pigment versus no pigment. However, as we have seen, the strain of *B. Bassiana* here studied has a flocculent type of growth and a slight pigment production, whereas the *B. effusa* strain has a downy growth and no pigment production. As pigment production has been noted by many to be a variable factor and to diminish or become entirely lost with time it would not seem a sound basis for differentiating species. Also there is no assurance that the strains now existing under the name *Bassiana* are indeed identical with that isolated by Bassi. Balsamo, who studied the original strain, noted the red coloration in the infected larvae. It is only after death that the mummy becomes covered with the white coating. Beauverie claims that with the red muscardine the color remains red. The larvae which we examined were covered with a white coating.

The spore measurements are within the limits of variation for the three species. It would seem that our chromogenic strain is definitely *effusa* and it certainly is indistinguishable from the strain which we studied under the name *Bassiana*, except in pigment production.

Our non-chromogenic strain might well answer to the description of the original *Bassiana* species in the type of growth and lack of pigment, in which characteristics it differs from the above three strains. The strain of *B. globulifera* studied has certainly not the macroscopic characteristics as first described for that species but variation in the type of growth for a given strain is common. The tendency to form a yellow pigment has always been attributed to this form and we have noted formation of glomerules. The two *densa* strains seem distinct both in spore size and shape, and in the slender conidiophores. The vitamin deficiency shown

by these two strains would also indicate a difference. We believe that *densa* is a distinct species, but that further studies should be made with the other strains here listed, particularly from the standpoint of the type of infection they cause and their virulence. Perhaps in this way a more definite conclusion might be reached as to both the number of distinct species involved and the variation that may occur within a single species.

Limber's strain is a distinct species and may be identical with Langeron's *B. Brumpti*.

SUMMARY

Two strains of *Beauveria* isolated from the wood borer *Nacerda melanura* were studied and compared with named species. The chromogenic strain appeared to be closely related to if not identical with the strains of *B. Bassiana* and *B. effusa* studied. Whereas the non-chromogenic strain seemed similar to the published descriptions of *B. Bassiana*, the two strains of *Beauveria densa* were identical, and are believed to constitute a distinct species. The strain of *B. globulifera* appeared different from the published descriptions of this species. It is, however, probably closely related to the *Bassiana* group, as it has been demonstrated that variation in growth characteristics may occur. Further studies are necessary, especially of the pathogenicity of these strains, before conclusions as to species differences can be drawn. A critical study of a number of species of *Tritirachium* should also be made in order to determine the validity of the genus. Sections of the infected larvae were studied and described.

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THE COPRINUS EPHEMERUS GROUP

MORTEN LANGE AND ALEXANDER H. SMITH

For some time we have been working on black-spored agarics, and on some problems involving the geographical distribution of these species. During this study we found it necessary to clear up the relationships between the American and the European species, eventually by studying them in culture. Special concern was given to the possible interfertility of American and European strains, and its bearing on our species concepts.

The Danish author undertook the necessary culture work, and found each American strain he raised to be interfertile with a group of European interfertile strains. The intrafertile groups thus defined were found to correspond to rather narrowly delimited species. A more detailed account of this work, including a discussion of the species concepts arrived at, is published elsewhere (Lange **1952**).

In the light of this study we venture to publish this contribution to a monographic treatment of the group in the hope that it will furnish those working in other parts of the world with species concepts which, when applied to the problem of distribution, will help to make that study more meaningful than has been the case in the past. A few species originally thought to belong here, but since excluded after a detailed study of their morphology, are included for convenience.

SURVEY OF DIAGNOSTIC CHARACTERS

The subsection is here in the main as defined by J. Lange (**1915, 1939**) using the absence of a universal veil and presence of pilocystidia as distinguishing characters. The species are found in two main habitats: on manure and on humid, black forest soil and mud. This difference in ecology proved to be a valuable and quite reliable diagnostic character, dividing the subsection into

a group of coprophilous and one of terrestrial species. The morphological characters show a rather great variability within each species, and both size and color can be used as distinguishing characters only after careful study. The size of the pileus is here in general recorded as the height just before expansion begins. The length and width of the stipe is taken on the fully mature fruit body. Ridgway (**1912**) was used as the standard for recording colors. It was found, also, that the microscopic characters vary a great deal, but still provide several important criteria of diagnostic value. Spore size and shape have been recorded with special care, and in addition to the usual maximum and minimum values we have generally indicated the variation in average size, as found by measuring a great number of spores of each of the available collections. The average ratio between length and width is a figure of some importance and is recorded for most species $\left(\frac{D}{d}\right)$. Almost all species have the spores slightly compressed. We have indicated this by figures for the difference between the two short diameters (d_1–d_2).

The basidia are dimorphic in some species, tri- or tetramorphic in others. Two-spored basidia have been found characteristic of some species, although a further study may reveal four-spored corresponding forms.[1] The presence or absence of pleurocystidia is an important character, especially in the coprophilous group, whereas differences in cheilocystidia often separate the species in the terrestrial group. Both pleurocystidia and cheilocystidia are best observed on buds which are on the point of expanding. Pilocystidia are always abundant on young buds and are of great diagnostic value. Their size is here recorded by the measurements of length, width at broadest point at base, and width just below apex (as close to apex as the sides can be considered to be subparallel). There are two types, one hyaline and thin-walled and the other with thick walls which vary from hyaline to brown. The two kinds intergrade. Such thick-walled bodies are often termed "setae," but they are truly pilocystidial in origin. We

[1] In the one case where two such corresponding forms were found to exist (*C. bisporus* and *C. congregatus*) they were found to be intersterile (Lange, **1952**) and hence are not regarded as identical.

prefer to designate them as *sclerocystidia* to differentiate them from the thin-walled type.

Clamp connections are present in all terrestrial species and in several of the coprophilous group. They are frequently hard to demonstrate on the fruit body, but form one of the best cultural characters, since they are easily demonstrated on the mycelium. Few other mycelial characters are of general importance, although great weight should be placed on the pairing reactions of the species. Both homothallic and heterothallic, bipolar and tetrapolar types are found within the subsection. Some of the species are amphithallic (Lange **1952**). In these species spores from the same cap give rise to both homothallic and heterothallic single-spore mycelia.

Strains in the same interfertility group will as a rule all be fully interfertile. Identical allelomorphs were, however, found in several cases, and also a few examples of reduced interfertility, due to other factors, but never correlated with any significant morphological differences.

TAXONOMY

As the subsection Setulosi (J. Lange **1915**) is used here it can be covered by the following amended description: Small to medium sized species; pileus fisso-sulcate or deeply grooved; no universal veil; partial veil absent (or rudimentary ?); deliquescing readily or tardily; pilocystidia present; pleurocystidia present or absent; cheilocystidia balloon-shaped or fusoid-ventricose; basidia di- or pleomorphic; spores brown to black (reddish in one species), generally more or less flattened; clamp connections present or absent.

The type species of the subsection is *Coprinus ephemerus*.

Singer (**1951**) has vaguely defined a subsection Auricomi, characterized by the presence of "setae" on the cap. Its type species, *C. auricomus* is not very well known, but the subsection is probably synonymous with Setulosi.

The true members of Setulosi form a group of closely related species which are frequently difficult to distinguish. We have arranged the species in stirpes, uniting those most closely related to

each other in small groups and arranging the whole in a sequence beginning with the most coprinoid, strongly deliquescing taxa. Those which are included here from other subsections and characterized by the presence of a universal veil made up of sphaerocysts apparently do not form a homogeneous group, and have here been arranged together with those of the Setulosi to which they seem to be most closely related.

KEY TO SPECIES

1. Coprophilous species, also growing on rotting straw; rapidly deliquescing 2
1. Habitat not as above 10
2. Sphaerocysts and clamp connections present; spores hexangular ..... *C. hexagonosporus*
2. Not as above 3
3. Sphaerocysts present, clamp connections absent; germ pore eccentric; spores narrow (5.2–7.5 μ), (if spores 7–9 μ broad in broadest view and buttons reddish see *C. curtus*) ..... *C. heptemerus*
3. Sphaerocysts absent on buttons 4
4. Clamp connections absent 5
4. Clamp connections present 8
5. Larger forms (buttons > 0.8 cm. high); spores medium to large (average length > 11.5 μ), germ pore eccentric 6
5. Small forms (buttons < 0.8 cm. high); spores small to medium sized (< 11.5 μ), germ pore almost apical 7
6. Basidia 4-spored; pileus clay color to ochraceous tawny ..... *C. congregatus*
6. Basidia 2-spored; pileus pale ochraceous tawny to ivory white ..... *C. bisporus*
7. Spores broad (D : d < 2); pleurocystidia present ..... *C. stellatus*
7. Spores narrow (D : d > 2); pleurocystidia absent ..... *C. pellucidus*
8. Spores long, average > 11.5 μ; pleurocystidia present 9
8. Spores short, average < 11.5 μ; pleurocystidia absent ..... *C. heterosetulosus*
9. Basidia 2-spored; thin-walled pilocystidia narrow, thick-walled pilocystidia (sclerocystidia) frequent ..... *C. Sassii*
9. Basidia 4-spored; thin-walled pilocystidia inflated; thick-walled pilocystidia (sclerocystidia) rare to absent ..... *C. ephemerus*
10. Carbonicolous species. Spores mitriform; pleurocystidia frequent; clamp connections present ..... *C. angulatus*
10. Terrestrial to sublignicolous species; pleurocystidia absent in many; clamp connections present; often tardily deliquescing 11
11. Spores vinaceous brown when fresh, turning pale chocolate color in KOH; pileus vinaceous brown in buttons ..... *C. fallax*

11. Not as above, spores typical for genus.....12
12 At least most of the cheilocystidia subglobose.....13
12. Cheilocystidia mostly bottle-shaped.....17
13. Pilocystidia subcapitate.....*C. plagioporus*
13. Pilocystidia obtuse to acute.....14
14. Spores long (> 11.5 μ), germpore eccentric.....15
14. Spore medium (< 12 μ), germpore eccentric to almost apical....16
15. Pileus and stipe flushed purplish; spores narrow (D:d > 1.9), almost black and impellucid.....*C. subpurpureus*
15. Pileus pallid to brown; spores paler; sclerocystidia on pileus frequent.....*C. sclerocystidiosus*
15. Not as in either of above choices—see *C. callinus*, the large spored forms.
16. Pilocystidia long (> 90 μ), mostly acute.....*C. callinus*
16. Pilocystidia shorter (< 90 μ) mostly obtuse.....*C. subimpatiens*
16. Pilocystidia short and acute, see *C. curysporus.*
17. Pilocystidia short, average < 60 μ.....18
17. Pilocystidia longer, average > 70 μ.....19
18. Spores in face view almost as broad as long, average < 11 μ *C. curysporus*
18. Spores longer in relation to widest view, average > 12 μ basidia 2-spored.....*C. amphithallus*
19. Fruiting body small; pilocystidia and cheilocystidia obtuse; spores > 10.5 μ long.....*C. subdisseminatus*
19. Fruiting body larger (buttons > 0.7 cm. high); cheilocystidia subacute; spores < 10.5 μ long.....20
20. Pilocystidia average < 90 μ; spores ovate, apiculus small.. *C. impatiens*
20. Pilocystidia long (> 110 μ); spores narrow (D:d > 1.9), apiculus prominent.....*C. hiascens*
20. Spores average < 8 μ; stipe and gills brown.....see *C. digitalis*

Section FARINOSI

1. **Coprinus heptemerus** nom. nov.

Coprinus curtus f. *macrosporus* Romagnesi (1941).

Illustrations: Lange 1952, Figs. 2, 9.

Pileus less than 1.0 cm high, subcylindric, narrow, rather pale, dull brown, pale "clay color," fissosulcate, deeply plicate when expanding, finally splitting at the edge and recurving; flesh very delicate and thin, odor and taste not distinctive; lamellae rather narrow and distant; stem whitish to hyaline, slightly brownish at the base; deliquescing.

Spores 10.8–15 × 4.4–7.5 μ, average [2] 11.5–13.9 × 5.2–6.7 μ, narrow, $\frac{D}{d} = 1.9–2.3$, somewhat flattened, $d_1–d_2 = 0.2–0.6\ \mu$, irregularly cylindric to narrowly ovate, rather pale chocolate color, suprahilar depression rather pronounced; germpore almost fully visible on abaxial side, often very large, up to 4 μ broad; basidia dimorphous, 20–33 × 10.5–13 μ, four-spored; paraphyses globose, about 18 μ broad; pleurocystidia absent; cheilocystidia balloon-shaped, about 25–50 μ broad; gill trama mostly of inflated elements, with an inner layer of fibrillose cells; pileus trama with a cuticle formed by a row of vesiculose cells mostly 20–30 μ broad; pilocystidia 50–122 × 9–23 × 2.5–4 μ, much inflated below, acuminate, sometimes furcate, hyaline, some incrusted, intergrading into large, globular, incrusted sphaerocysts; clamp connections absent in all tissues and on the mycelium, which in culture has a very pronounced dark brown color.

Homothallic, fruiting abundantly in culture within seven days after inoculation.

Habit, habitat and distribution: Subsolitary on dung, France *, Denmark *, Sweden * and U.S.A.*

Observations: This fungus was first described by Romagnesi (**1941**) as a form of *C. curtus*, from which it is clearly distinguishable by the lack of reddish colors, acuminate cystidia, pale spores with strongly eccentric germpore and several other characters. When the sphaerocysts are washed away it bears some likeness to *C. stellatus* and *C. pellucidus* but it is probably not intimately related with the species in the section Nudi. We have seen several collections by Brooks from Michigan, it has been found twice in Denmark and twice in Sweden, and we have checked the authentic culture of *C. curtus* f. *macrosporus* in Baarn. The latter was found to be identical with our own material in all details and should be considered as the type.

[2] Indicates the smallest and the largest average spore size found when measuring spores from several different collections. For many species a very large variation is recorded.

* The asterisk indicates that specimens from that country have been cultured and in heterothallic species found interfertile with other strains of the species.

2. COPRINUS HEXAGONOSPORUS Josserand (1948).

Illustrations: Josserand 1948, Fig. 1; Smith 1948, Fig. 9; Lange 1952, Figs. 1, 9.

Pileus cylindric at first, 0.5–2.0 cm high, very finely pubescent over disc, disc "fuscous," marginal area pallid to "avellaneous," at maturity the disc often tinged "army brown," surface closely folded-striate before expanding, becoming broadly convex to plane and plicate-striate before maturity; flesh very delicate and thin, odor none, taste mild to slightly bitterish; lamellae free but attached at apex of stipe, close, becoming subdistant, whitish pallid, soon avellaneous and then black, narrow and equal, soon deliquescing; stipe 3–5(–10) cm long, 0.5–1(–2.5) mm thick, equal, hollow, very fragile, sparingly pubescent at first from projecting caulocystidia, densely pubescent above, white or whitish, hyaline at first but soon sordid brownish over lower two-thirds, base often inserted on the substratum.

Spores black in deposit, 8.6–14.5 × 5.4–8.3 μ, aver. 10.9–12.6 × 6.05–7.3 μ, distinctly flattened, d_1–d_2 = 0.75–1.15 μ, subelliptic in side view, angular in face view (the sides parallel and angled to apex and base), pore distinctly to very much eccentric (sometimes appearing circular in face view of spore), blackish brown, black in KOH; basidia four-spored, rarely with a few two-spored basidia intermixed on same pileus, 20–34 × 8–11 μ, more or less trimorphic; paraphyses 18–22 × 9–16 μ; pleurocystidia rare to absent, 60–90 × 20–35 μ, becoming more elongated and narrower, readily collapsing; cheilocystidia similar to pleuro-cystidia or more vesiculose, 40–90 × 35–45 μ; gill trama narrow and of very loosely interwoven cells; pileus trama very thin but hyphae filamentose, those in the dark discal area dark sordid brown (almost vinaceous brown) when revived in KOH; cuticle of vesiculose cells, pilocystidia 50–120 × 9.5 μ, inflated below, neck subcylindric, apex more or less distinctly capitate (width respectively 9.5–22 μ, 4–10 μ and 4–13μ), hyaline or slightly brownish below, typical sclerocystidia not present but pilocystidia intergrading into incrusted globose sphaerocysts on the surface, these mostly 20–30 μ broad; clamp connections present on hyphae at base of stipe and on mycelium.

Heterothallic and tetrapolar.

Habit, habitat and distribution: Solitary or gregarious on dung and rotton straw, France*, Denmark* and U.S.A.*

Observations: This very outstanding species was first mentioned by Pennington (**1918**) as a form of *C. ephemerus*. It is

recognizable macroscopically by its dark fuscous to vinaceous brown apex, but the microscopic characters are necessary to clearly distinguish it from *C. Sassii*. Josserand (**1948**) noted the sphaerocysts on the pileus. However, these are generally very scanty except on the very young bud. The species is probably the best link between the Nudi and Farinosi. The color of the pileus and type of pilocystidia indicate relationship with *C. curtus*.

Stirps *Sylvaticus*

3. COPRINUS SILVATICUS Peck (1870).

Coprinus tardus Karsten (1883).

Pileus 1–4 cm high, broadly elliptic-ovate, then broadly convex and finally almost plane, the edge sometimes recurving, finely pubescent, soon almost glabrous, fisso-sulcate to the smooth disc, disc and back of striae dark brown ("cinnamon brown" "russet"), darker when very moist, paler in some forms, appearing somewhat viscid when moist; flesh concolorous with pileus, pelliculose except in the disc, taste and odor none, tardily deliquescing; lamellae distinctly brownish when young, black when spores ripen, crowded to subdistant, narrow, adnate to almost free; stipe 1–8 cm × 0.2–2.5 mm, whitish to yellowish brown.

Spores 10.3–15.8 × 6.2–10.0 μ, flattened, d_1–d_2 = 0.6–1.15 μ, broad, D : d = 5–1.6 μ, broadly ovate or almost circular in face view, slightly inequilateral in side view, with low warts and ridges covered by a more or less distinct wrinkled hyaline epispore, apiculus prominent, blackish brown to almost impellucid, germpore apical; basidia 20–46 × 9–11.5 μ, four-spored, rarely with some few two-spored intermixed, tetramorphic, long type narrow, much protruding; paraphyses inflated, pleurocystidia absent; cheilocystidia bottle shaped, 38–64 × 16–18 × 5–8 μ, in a rather dense band; pileus trama brownish, cuticle of vesiculose cells, pilocystidia very large, 75–135 × 15–25 × 4.5–9.5 μ, sphaerocysts present but seldom numerous, 20–35 μ broad, brownish with wrinkled walls.

Heterothallic, clamp connections present on mycelium.

Habit, habitat and distribution: Subfasciculate to gregarious, on rich soil in woods and gardens. We have seen material from U.S.A., Canada, Finland, and Denmark *, but also on record under different names from several European countries in addition to the above.

Observations: This species is easily recognized by the large rough spores, the stature which reminds one of *C. micaceus*, and in somewhat the same color pattern as the latter. Various forms have been described as follows: with almost glabrous spores, with very few pilocystidia, and with pallid colors. The species is often called *C. tergiversans* Fr. sensu Ricken, but as pointed out by J. Lange (**1939**) the Friesian description hardly fits this plant. The concept of Peck's species is well based on the emended description of the type (Smith **1948**). In addition, we have studied the type of Karsten's *C. tardus* and found it identical in all characters. The sphaerocysts are not mentioned in the current descriptions, but have been demonstrated on both American and European material. The spores of this species are very difficult to germinate, and no American material has been studied in culture. However, we feel quite certain that the American and European material studied belongs in one species on the basis of resemblance in morphological characters.

Section Nudi: Subsection Setulosi:

Stirps *Ephemerus*

4. **Coprinus Sassii** nom. nov.

Coprinus ephemerus f. *bisporus* Sass (1929).

Illustrations: Lange 1952, Figs. 2, 10.

Pileus 0.8–2(–2.6) cm high, narrowly conic, expanding to obtusely conic, the margin finally recurving and splitting, surface pruinose at first but soon almost glabrous, moist, plicate-striate to the disc but disc not differentiated by a sharp line, "russet" to "cinnamon brown" with a slight purplish flush when young, "mummy brown" when spores mature, the disc retaining a tawny tinge; flesh very thin, soft, delicate, odor and taste none; lamellae very narrow, free or just reaching the stipe, rather crowded, pallid, edge whitish but finally black over all as they deliquesce; stipe 4–8 cm long, 2–3 mm thick, hollow, fragile and very delicate, white or more frequently flushed with color of the pileus from base up.

Spore deposit black, spores 12.8–20 × 7.9–11.0 μ, average 15.2–17.3 × 8.7–9.8 μ, $\frac{D}{d} = 1.7–1.75$, almost terete, broadly ovate in

face view, broadly elliptic-ovate in side view, dark brown, blackish brown in KOH, germpore distinctly visible on abaxial side, large, about 2.5 μ broad; basidia 18–38 × 9–12.5 μ, constantly two-spored, dimorphic, the short type often considerably broader than the long one; paraphyses subglobose, 12–18 × 18–25 μ; pleurocystidia scattered to abundant, vesiculose, 50–90 × 15–55 μ; cheilocystidia similar but somewhat shorter, mostly 30–60 × 30 μ; gill trama scanty, hyphae about 3 μ broad, a few of them with clamp connections, rusty brown near cap trama which is made up of narrow elements, rusty brown in KOH; cuticle of subglobose cells with rather thick walls, diam. 20–35 μ, pilocystidia 30–100 × 4.5–16 × 1.5–5 μ generally rather thick-walled and brownish from the base up more or less, some of them typical sclerocystidia and all of them narrow, pedicellate to sessile, subventricose, acuminate, apex acute (subacute in one American coll.), the base frequently incrusted with hyaline material, more rarely pilocystidial bodies are seen without a neck, but with a long pedicel.

Single spore mycelia from the same pileus always of two types, with and without clamp connections, the former homothallic, the latter heterothallic, bipolar ("amphithallic" bipolar, cfr. Lange **1952**).

Habit, habitat and distribution: Gregarious on horse-dung and on rotten straw, U.S.A., Denmark *, Sweden *.

Observations: The material described here evidently belongs in what Sass (**1929**) called *C. ephemerus* f. *bisporus*. We have studied the type in the University of Michigan Herbarium, and found it fully identical with our collections. In addition, the cultural characters were as described by Sass. It is different from *C. ephemerus* (whichever concept of the latter one chooses to accept) by the characteristic pilocystidia and the outstanding color. In stature and color it resembles *C. hexagonosporus*. We have a single American collection, from Kansas, of a four-spored *Coprinus* probably closely related to *C. Sassii* but with more pronounced purplish colors and smaller spores. Until more collections have been made, we hesitate to consider it the four-spored form of *C. Sassii*. The two-spored basidia of *C. Sassii* are a genetically constant feature as is evidenced by their occurrence through several generations in culture. The sparingly occurring cystidia without necks might indicate a relationship to species with sphaerocysts.

5. COPRINUS EPHEMERUS Bull. ex Fr. sensu Locquin (1947).

? *Coprinus proximellus* Karsten (1879).

Illustrations: Locquin 1947, Fig. 1; Lange 1952 Figs. 2, 10.

Pileus up to 2 cm high, generally 1–1.3 cm but also much smaller, buttons narrow but becoming broadly conic, the margin recurving and splitting in age, fisso-sulcate, plicate-striate when maturing, disc pale to dark "cinnamon brown" or slightly more vividly colored, somewhat shining when moist; flesh thin, distinctly bitterish, rapidly deliquescing; gills close to subdistant, up to 36 reach the stipe, narrow, very narrow behind, almost free, pallid, blackening from the spores, edge whitish; stipe up to 7 cm long and 3 mm thick, white, slightly thickened and strigose below, hollow, caulocystidia present.

Spores black in deposit, very variable in size, 8.6–16.7 × 5.3–8.5 μ, average 11.35–15.4 × 6.1–7.6 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.8–2.05$, slightly flattened to almost terete, $d_1–d_2 =$ 0.0–0.7 μ, mostly 0.15–0.5 μ, ovate in face view, elliptic-oval in side view, moderately dark brown to almost black, black in KOH, germpore more or less visible in abaxial side; basidia 20–33 × 11–13 μ, four-spored, mostly dimorphic; paraphyses subglobose-pyriform, 13–20 μ broad; pleurocystidia abundant, 60–120 × 22–45 μ, vesiculose, more or less pedicellate; cheilocystidia similar or shorter, subglobose, 20–60 × 20–30 μ, a few elongated-ventricose cheilocystidia noticed on most specimens, generally about 55 × 15 μ; gill trama scanty, of thin, sometimes clamp-bearing hyphae with additional more inflated subhymenial elements; pileus trama mostly of inflated elements, 12–14 μ broad; cuticle of globose to subglobose cells 25–30 μ across, in the grooves more oblong, and then up to 70 × 25 μ; pilocystidia 33–94 × 9–19 × 3.5–8 μ, more or less ventricose, neck subcylindric to somewhat tapering, apex obtuse, hyaline or faintly brownish below; sclerocystidia absent.

Heterothallic, bipolar, the mycelium with numerous clamp connections.

Habit, habitat and distribution: Solitary or gregarious, on cow dung, usually after the droppings have aged about a month or more and persisting at times on very old washed-out substratum. It occurs rarely on rotting straw, Denmark *, Norway *, Sweden *, France, U.S.A.*

Observations: This species is distinguished from other large-spored species of the group by the presence of clamp connections,

bipolarity, and other cultural as well as microscopic characters, but it can often be recognized macroscopically. It is evidently the species studied in culture by Sass (**1929**) and by Quintanilha *et al.* (**1941**). It was described very well by Locquin (**1947**) who noted the bitter taste and the affinity for cow dung. The "correct" application of the name *C. ephemerus* is, in our opinion, an almost insoluble problem, and perhaps the name should be discarded. Fries noted (**1874**) that the name was used for a group of species. Brefeld, Karsten and Schroeter used the name for a small-spored species, whereas Cooke and all more recent authors have used it for a large-spored species. There is little doubt that Karsten called this species *C. proximellus,* though we have not been able to demonstrate the clamp connections on his type material. Karsten's description is, however, entirely insufficient. We follow the concept of Locquin not only because we thus are in accordance with the majority of later authors, but particularly on the strength of the original Friesian description (Fries **1821**), indicating relatively large size and brown colors.

6. COPRINUS HETEROSETULOSUS Locquin (1947).

Illustrations: Locquin 1947, Figs. 2, 3; Lange 1952, Figs. 3, 10.

Pileus rarely more than 0.7 cm high, rather broad and obtuse, densely cystidiose when young, fisso-sulcate, then plicate-striate, striae frequently somewhat wrinkled, disc dark brown "Saccardo's umber," the striae more or less flushed with this color, margin appressed against the stipe at first, pale, turning blackish and splitting when spores ripen; flesh delicate, without taste, soon deliquescing; lamellae narrow, close to subdistant, often only 16 reach the stipe, almost free, pallid brownish, almost black when spores ripen; stipe about 1–3 cm long, 1.0 mm thick, hyaline whitish, flushed with the color of the pileus at the base, densely pubescent when young, hollow.

Spores almost black in deposit, 8.1–12.8 × 4.8–6.6 μ, average 9.6–11.15 × 5.4–6.0 μ, fairly narrow $\left(\frac{D}{d} = 1.8\text{–}2.05\right)$, slightly flattened to almost terete, ovate in face view, elliptic in side view, germpore slightly eccentric, hardly visible on abaxial side, dark brown to blackish brown, fuscous to black in KOH; basidia four-spored, 20–35 × 8–9.5 μ, trimorphic; paraphyses subglobose, 14–

17 μ broad; pleurocystidia absent, cheilocystidia globose, 15–30 μ across, elongated-ventricose types rare to scattered; gill trama of thin clamp-bearing hyphae, with inflated subhymenial elements; trama of cap of inflated elements, 10–30 μ broad, mixed with some thinner, dark brown hyphae; cuticle of globose cells, more oblong, up to 75 μ long in the grooves; pilocystidia 30–105(–140) $\times$ 7.5–21 $\times$ 3–6(–9) μ, often distinctly ventricose, base frequently brownish, neck somewhat tapering, apex subobtuse to almost acute; sclerocystidia rather abundant to rare, not seen in some caps, when present small, brown, thick-walled and irregular, rarely more than 33 $\times$ 8.5 $\times$ 3 μ.

Heterothallic, tetrapolar, clamp connections abundant to scattered on the mycelium.

Habit, habitat and distribution: On dung, often when rather old and washed out, France *, Denmark *, Norway *, Sweden *, U.S.A.* (Michigan and Washington).

Observations: This species is well characterized through its microscopic characters, especially when sclerocystidia are present. It is the only small-spored species in the coprophilous series with clamp connections. Locquin (**1947**) suggests that the species which Josserand (**1933**) called *C. ephemerus* belongs here, and we have actually had material from Josserand supporting this. None of the ancient descriptions fit this plant exactly, and it has probably been overlooked. It may possibly be *C. ephemerus* sensu Bisby, Buller and Dearness (**1929**).

7. COPRINUS CONGREGATUS Bull. ex. Fr.

Illustrations: Lange 1952, Figs. 2, 11.

Pileus 0.2–3.0 cm high, subellipsoid and rather narrow at first, expanding to conic campanulate with a flaring margin, subpubescent from cystidia, soon appearing almost glabrous, moist, sulcate-striate to the smooth disc, the disc and the backs of the striations "clay color" to "ochraceous tawny," marginal area "pinkish buff," finally dark gray over striate portion, sordid alutaceous over the disc; flesh very soft and fragile, entire cap deliquescing in humid weather, in dry weather only the gill edges deliquescing, no distinctive odor or taste; lamellae narrowly adnate, close (about 26 reach the stipe), narrow, whitish to "cartridge buff" when young, darkening to avellaneous and finally more sordid than "wood brown," edges white until deliquescence begins; spores ripening

from margin upwards or almost simultaneously; stipe white, 2–8 cm long, 0.6–3 mm thick, equal, hollow, pubescent from caulocystidia, strigose below, sometimes with a slight collar-like ridge and somewhat rooting.

Spores black in deposit, 9.0–14.7 × 5.4–7.6 μ, average 10.8–13.0 × 6.0–6.95 μ, moderately broad, $\frac{D}{d} = 1.75–1.95$, slightly flattened to almost terete, $d_1–d_2 = 0.0–0.5$, dark brown, fuscous to black in KOH, germpore distinct, slightly to very eccentric, always somewhat visible on abaxial side; basidia 18–30 × 9–11 μ, four-spored, di- or trimorphic; paraphyses subglobose, mostly 15–18 μ broad; pleurocystidia abundant, 70–120 × 20–50 μ, vesiculose, if more elongated the apices obtuse; cheilocystidia similar or somewhat shorter, subglobose, 30–70 × 20–30 μ, elongated-ventricose individuals rare to absent; gill trama thin, hyaline, hyaline to sordid brownish in KOH; cuticle of subglobose cells, 25–30 μ broad, oblong in the grooves where they are up to 65 μ long; pilocystidia 48–106(–150) × 7.5–17 × 4.5–12 μ, hyaline, subcylindric, slightly ventricose, apex obtuse.

Heterothallic, bipolar, the ability to form fruit bodies on diploid mycelia in some strains depending on a special gene, clamp connections absent on mycelium and in fruit body.

Habit, habitat and distribution: Solitary, gregarious or more often subfasciculate, on dung piles, on bits of manure in flower beds, hothouses, on soil mixed with kitchen refuse or on rotten straw, Sweden *, Denmark *, Germany, Iceland *, U.S.A.*

Observations: From its cultural characters this species is evidently identical with that studied by Brunswik (**1924**) under the name *C. ephemerus*, but it is hardly adequately described in recent literature. It is, however, in all probability the species Fries mentioned under *C. congregatus*. It shares the pale colors, grows subfasciculate, and Fries knew it from hothouses. In view of the inconsistent use of the name it might be preferable to rename the species, but this point of view could be taken for so many of the older species that one hesitates to accept it except for the most flagrant cases. If future work should show that *C. bisporus* is the next older name, and that *C. congregatus* sensu L. & S. is merely a four-spored form, the species epithet would be a most unfortunate choice.

8. COPRINUS BISPORUS J. Lange (1915).

Illustrations: J. Lange 1939, Plate 160, Fig. E; M. Lange 1952, Figs. 2, 11.

Pileus 0.5–2.5 cm high, subellipsoid and rather narrow at first, expanding to conic-campanulate, cystidiose, soon almost glabrous, sulcate-striate to the disc, pale ochraceous tawny to ivory white, the disc slightly darker ("clay color") or paler buff ("pinkish buff"), eventually fading to whitish, flesh thin, without distinctive odor and taste, deliquescing; lamellae narrow, crowded, adnexed to the top of the stipe or almost free, pallid when young, darkening from the margin upwards, margin white until deliquescing; stipe 2–10 × 0.1–0.3 cm, white-strigose below, rooting.

Spores black in deposit, 9.5–14.6 × 5.2–7.4 μ, $\frac{D}{d} = 1.8–1.95$, $d_1–d_2 = 0.15–0.55\ \mu$, dark brown, germpore distinctly eccentric and always somewhat visible in face view; basidia 18–33 × 6.5–7.5 μ, two-spored, di- to trimorphic; paraphyses subglobose-pyriform, about 16 μ broad; pleurocystidia rare, absent in most collections; cheilocystidia vesiculose, 20–60 × 15–30 μ, elongated-ventricose individuals scattered to absent; gill trama fibrillose, of very narrow hyphae and a few inflated subhymenial elements; pileus trama mostly of inflated elements, hyaline; cuticle of globose-subglobose cells 18–35 μ broad, oblong in the grooves and up to 60 × 25 μ; pilocystidia subcylindric, slightly inflated below, apex obtuse, 55–140 × 8.5–22 × 4–9.5 μ.

Homothallic, without clamp connections on the mycelium or in the fruit body.

Habit, habitat and distribution: On dung, often a mixture of dung and soil, subfasciculate, Denmark *, Sweden *, Greenland *, U.S.A.

Observations: This species is evidently that depicted by J. Lange (**1939**). It has the same growth form, the same pale colors and the same type of pilocystidia. It is closely related to *C. congregatus*, but differs in bispored basidia, which is a constant character in culture, smaller basidia, paler colors, and more distinct subfasciculate growth. In culture the two species are further distinguished by a pronounced brownish color of the mycelium of *C. congregatus*. Non-fruiting single spore mycelia of *C. bisporus* are intersterile with haplonts of *C. congregatus* (Lange **1952**).

9. COPRINUS STELLATUS Buller (1929).

Illustrations: Lange 1952, Figs. 3, 12.

Pileus 0.15–1.0 cm high, short cylindric-conic, the margin flaring and splitting at maturity, wrinkled-striate, becoming plicate, disc smooth, "ochraceous tawny" to "tawny olive," marginal area soon paler, becoming gray to black when spores mature, surface at first densely cystidiose from very small cystidia, glabrous in age; flesh very soft and delicate, no odor or taste, rapidly deliquescing; lamellae narrow and close, distant on very small specimens, free, pallid, becoming dark fuscous to black from the spores, edges white until deliquescence begins; stipe 1–5 × 0.05–0.25 cm, cylindric, densely cystidiose when young, base equal, sometimes rooting, sometimes slightly inflated, somewhat strigose, hollow, whitish or hyaline whitish, in some specimens brown (even as dark as bay brown) below.

Spores black in deposits, 7.9–13.3 × 4.4–6.4 μ, averages 8.8–12.1 × 4.9–6 μ, almost terete, moderately broad to rather narrow, $\frac{D}{d}$ = 1.75–2.05, blackish brown in water mounts, black in KOH, much paler if not fully mature, germpore apical; basidia four-spored, 16–30 × 7.5–9(10) μ generally dimorphic, paraphyses almost globose, 17–24 μ broad, much narrowed in young stages; pleurocystidia abundant to scattered 70–130 × 15–40 μ; cheilocystidia rather variable, forming a broad band or more scattered, similar to pleurocystidia or some of them subglobose, always with some short, ventricose, acuminate ones between the vesiculose individuals, the fusoid-ventricose type seldom more than 30 μ long; gill trama mostly fibrillose, hyaline to pale brownish, pileus trama of short, somewhat inflated elements; cuticle of subglobose to globose cells 15–35 μ broad, not much longer in the furrows; pilocystidia very numerous and rather uniform in size and shape, hyaline or faintly brownish at base, 25–65 × 7–14 × 2.5–5 μ, acuminate.

Homothallic, clamp connections absent on mycelia and in fruit body.

Habit, habitat and distribution: Solitary, gregarious, or more rarely subfasciculate, on dung. Probably world-wide in distribution. We have seen specimens from Denmark *, England *, Iceland *, India, Sweden *, and U.S.A.*

Observations: *C. stellatus* is best characterized through the very abundant, small pilocystidia. Our material has shown some varia-

tion in spore size, and size and shape of the various cystidia, but none of these differences have been constant in culture. We have never seen the pleurocystidia as long as indicated by Buller, but for the rest, his description (in Bisby, Buller and Dearness **1929**) fits our plant excellently. It is highly probable that this is *Agaricus subtilis* Fries, but we deem it better not to revive this name.

Stirps *Pellucidus*

10. COPRINUS PELLUCIDUS Karsten (1883).

Pileus 0.15–0.7 cm high, obtuse to convex, plicate-folded, splitting as it expands and hence torn into segments, segments horizontal or slightly recurved before finally deliquescing, young buds rather distinctly striate, disc dark ("cinnamon brown"), rarely paler, striae on young bud flushed with this color, but whole marginal area soon paler before turning blackish gray from the ripening spores; flesh membranaceous and delicate, slightly bitterish, odor faintly rankish, often but tardily deliquescing; lamellae narrow, close, 16–35 reach the stipe, free, chocolate brown to blackish brown from the ripe spores, edges white; stipe whitish to pale brownish hyaline, 0.5–4 × 0.05–0.15 cm, filiform, densely pubescent when young, sometimes slightly inflated at base.

Spores 6.4–11.4 × 3.1–5.1 μ, averages 7–10.5 × 3.4–4.8 μ, terete, narrow $\frac{D}{d} = 2.0–2.15$, shortly cylindric to narrowly ovate in face view, similar or slightly reniform in side view, cocoa-color in water when fresh, rarely darker, chocolate color in KOH; germpore apical, apiculus very indistinct; basidia mostly 16–26 × 6–8 μ, four-spored, di- and trimorphic; paraphyses small, almost globose, 10–16 μ broad; pleurocystidia not seen; cheilocystidia balloon-shaped, up to 40 μ broad, sometimes mixed with a rather large number of acuminate cystidia, the latter type even dominant in some collections; gill trama fibrillose with some inflated elements, pileus trama similar, frequently rather dark brown; cuticle of subglobose to globose cells, 15–28 μ broad; pilocystidia variable in different specimens, and also sometimes on the same pileus, 20–125 × 5–11.8 × 2–10 μ, acuminate, hyaline, rarely brownish below.

Homothallic, clamp connections absent on mycelium and in fruit body.

Habit, habitat and distribution: Solitary (in culture sometimes subfasciculate) on dung, Finland, Norway *, Denmark *, U.S.A.*

Observations: The species is best characterized by the small narrow spores. Karsten (**1883**) gives the spore size as 7–9 × 4 μ, in good agreement with what is recorded above. We have seen the type collection, which is made up mostly of small bits of dung, and on which the fungus has become almost obliterated. It was, however, possible to find a few spores on a fragment of the stem, and they were typically like those described here, and we believe we are fully justified in accepting the Karstenian name for this species. *C. pellucidus* would seem to merit further study in order to determine whether the differences recorded in the size of spores and pilocystidia would establish the presence of additional taxa.

Stirps *Angulatus*

11. COPRINUS ANGULATUS Peck (1874).

Coprinus Boudierii Quélet.

Illustrations numerous: i.a. Konrad & Maublanc 1929, Plate 37, Fig. 11.

Pileus 0.4–2.5 cm high, pubescent when young, rather broad and obtuse, at first acorn-shaped, then ovoid to hemispheric, becoming expanded, the margin recurving, wrinkled striate-sulcate, dark tawny to "Mars brown" all over when young, sometimes with a faint purplish tint, marginal area fading to "ochraceous tawny" before it becomes lead-color to blackish from the spores; flesh thin and fragile, taste and odor not distinctive, strongly deliquescing in humid weather; lamellae close, becoming subdistant, moderately broad, pallid, becoming black, the edges white-fimbriate until deliquescing; stipe comparatively short, 1–5 × 0.1–0.3(–0.4) cm, pubescent, whitish, often watery brownish within.

Spores black in deposits, 7.3–11.6 × 4.5–8.1 μ, averages 8.05–10.25 × 5.85–6.5 μ, much flattened, d_1–d_2 = 0.85–2.5 μ, mitriform in face view, obliquely ovate-elliptic in side view, but varying much in shape inside this general pattern, dark brown to almost black, dark bistre brown to black in KOH, germpore apical, apiculus very small, often difficult to demonstrate; basidia 16–33(–39) × 7–10 μ, four-spored, trimorphic, all but the shortest ones pedicellate, ventricose, the longest slightly narrowed above midportion; paraphyses voluminous, 14–18 × 8–12 μ; pleurocystidia numerous to scattered, 55–125(–150) × (20–)40–70 μ; cheilocystidia dimorphic, on the covered part of the smaller gills similar to short

pleurocystidia, on the free gill-edges subglobose or ventricose bottle-shaped, the latter type being about 25–50 × 10–15 × 3.5–4 μ; gill trama of rather short, broad elements, subhyaline; pileus trama similar but tawny to cinnamon brown; pilocystidia 30–117 × 7–23 × 6.5–9.5 μ, somewhat inflated, mostly subacuminate (more obtuse types also seen), hyaline or brownish at the base, intergrading to more narrow, thick-walled sclerocystidia, the latter present in most specimens but in very variable number, in one collection measuring 60–82 × 6.5–9 × 3–3.5 μ, smaller in some collections.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: On burnt-over areas, attached to small fragments of charcoal, gregarious to solitary. We have seen material from U.S.A.*, Austria *, Denmark *, France *, Greenland *, Sweden *, and it is on record from additional countries.

Observations: The species is readily distinguished on the spore characters and on the peculiar habitat. Smith (**1948**) studied the type of Peck's species, making the status clear. If a velate species with the same type of spores really exists, as supposed by Josserand (**1944**), it should be described under a new name.

Stirps *Fallax*

12. **Coprinus fallax** sp. nov.

Statura *C. subpurpureo* et *C. plagioporo* similis. Sporae congestae atro-rubescentes, magn. 11–14 × 6–7.5 μ; basidia dimorpha, magn. 20–24 × 7–8 μ; pilocystidia magn. 20–30 × 100–120 μ.

Fibulae praesentes.

Ad terram caespitosus.

Typus in Dexter, Michigan sub numero 32096 ab A. H. Smith lectus, in Herb. Univ. Mich. depositus.

Pileus about 10 mm high before buttons expand, obtusely conic, expanding to convex or broadly conic, margin appressed to stipe at first, surface moist and hygrophanous, plicate-striate to the watery disc, soon splitting in the creases, color "walnut brown" to "Rood's brown" on disc, margin about "fawn color" (dark to pale vinaceous brown); flesh membranous and delicate; lamellae adnate to apex of stipe or barely touching it, subdistant, narrow, edges soon deliquescing and discolored, faces about concolorous with pileus; stipe 2–3.5 cm long, about 1 mm thick, equal,

hyaline, white or at base slightly discolored, surface faintly fibrillose.

Spores 11–14 × 6–7.5 μ, near "pecan brown" in water mounts of fresh spores, reddish in deposits, pallid in KOH mounts of dried material but soon changing to chocolate gray-brown, in side view slightly inequilateral, face view ovate, germpore more or less eccentric but very difficult to demonstrate on any but darkest spores; basidia dimorphic, 20–24 × 7–8 μ, hyaline, four-spored; paraphyses vesiculose, about 20 μ broad; pleurocystidia not seen; cheilocystidia (all collapsed); pileus trama with a cellular cuticle about 1 cell deep, the cells thin-walled and hyaline in KOH; pilocystidia numerous, (15–)20–30 × 100–120 μ (or shorter if neck is short), ventricose at base and with a long neck terminating in an obtuse apex, thin-walled, hyaline in water mounts; small hyaline sclerocystidia very rare to absent; clamp connections present on mycelium around base of stipe; caulocystidia similar to pilocystidia or more elongated.

Not cultured.

Habit, habitat and distribution: Cespitose on soil in a lumber yard, Dexter, Mich., May 24, 1949 (Smith—*32096*). It is known only from the type locality.

Observations: The vinaceous brown colors are distinctive in *Coprinus*, but other species with them are known. However, the color of the spore deposit is aberrant in the same degree as the spore color of the *Psathyrella sarcocephala* group is aberrant for that genus. The reaction of the spores in KOH reminds one of the reaction of the spores of some tropical species of *Psathyrella* in the same medium. In the character of deliquescing, in the characters of the hymenium, and in aspect the fungus clearly falls in *Setulosi* of *Coprinus*. The reaction of the spores in KOH definitely indicated relationships here rather than in *Bolbitius*.

Stirps *Plagioporus*

13. COPRINUS PLAGIOPORUS Romagnesi (1941).

Coprinus ephemerus f. *saturatus* J. Lange (1939)

Illustrations: Romagnesi (l.c.) Fig. 6; Lange 1952, Figs. 4, 13.

Pileus 0.5–1.2 cm high before expanding, buds rather broad, margin adpressed to stem, wrinkled-fisso-sulcate, plicato-striate when expanding, transversely rugulose in the grooves, at last

revolute, color rather variable, brownish, generally with a faint reddish hue, the disc being always darker than the sulcate part, sometimes as dark as "bone brown," but frequently paler, "wood brown" or even dull "vinaceous buff," the striae on the expanding cap slightly paler than the disc, paler (pale "ochraceous buff") between the striae, finally darkening as the spores ripen, densely cystidiose at first, often beaded with drops; flesh of cap delicate and thin, concolorous, taste slightly bitterish, odor none; gills moderately close, 28–36 reach the stipe, one to three tiers of lamellulae, rather narrow, broadest (about 2 mm) above but almost free, soon black from the spores maturing almost simultaneously over the whole gill; stipe 2–6 × 0.1–0.2 cm, slightly swollen below, hollow, whitish, more rarely flushed with the color of the cap at base, inside and outside, densely cystidiose; gills and (under humid conditions) the entire cap, deliquescing.

Spore print almost black, spores 9.1–14 × 5.4–7 μ, averages 10.9–12.05 × 6–6.4 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.75–2.0$, slightly flattened, $d_1–d_2 = 0.3–0.6\ \mu$, oval in face view, elliptic with the adaxial side slightly flattened in side view, fuscous to almost black, with germpore moderately eccentric, always visible on the abaxial side; basidia 18–35 × 8.5–9.5 μ, four-spored tetramorphic; paraphyses numerous, subglobose-pearshaped, 15–20 × 23–33 μ; pleurocystidia not seen; cheilocystidia numerous, subglobose-balloon-shaped 18–45(–65) μ across, elongated-ventricose types (similar to pilocystidia) rare to scattered but observed in all specimens examined; gill trama sordid brown to hyaline; pileus trama paler or darker brownish, mostly of inflated elements; cuticle of globose to subglobose cells, mostly 25 μ broad, more oblong in the furrows, up to 90 × 50 μ; pilocystidia 43–103 × 9–20 × 5–12.5 μ, moderately inflated below, subcapitate, neck 4–10 μ broad; sclerocystidia rare to absent, when present similar to other pilocystidia but smaller, dark brown and very thick-walled.

Amphithallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Solitary or subfasciculate, mostly on small sticks and the like, but also truly terrestrial, on black muck, etc., Denmark *, France, Sweden *, U.S.A.

Observations: This species is easily recognizable by the characters of the pilocystidia, which are very constant in size and shape. Except for the absence of pleurocystidia, our plants correspond well with the original description by Romagnesi (**1941**),

and so we have assigned them here. We have studied a spore print from the collection of J. Lange and found indications that *C. ephemerus* f. *saturatus* J. Lange covers this species (germpore moderately eccentric, spores dark, averaging 11.75 × 6.05 μ.).

14. COPRINUS SUBPURPUREUS Smith (1948).

Illustrations: Smith l.c. Fig. 25; Lange 1952, Figs. 4, 13.

Pileus about 1.5 cm high, cylindric to ovoid young and expanding to campanulate before the spores are shed, becoming nearly plane or even recurving when gills deliquesce, finely pruinose-pubescent when young, soon almost glabrous as cystidia collapse, folded-striate at first, broadly plicate-striate at maturity, disc dark vinaceous brown ("army brown," "natal brown" to "Benzo brown"), "light brownish drab" toward the paler ("light cinnamon drab") broad striate marginal area, in age "dark purple drab" over disc and dark gray to blackish over margin, vinaceous brown tinge often remaining over the backs of the striations; flesh very thin and fragile, delicate, no odor or taste; lamellae narrowly adnate, close, becoming subdistant, narrow, near "tilleul buff," darkening to black over all before the edges deliquesce; stipe 3–10 × 0.05–0.25 cm, equal, dull lilac umber young, rarely pallid, becoming paler in age, densely pruinose-pubescent but soon glabrescent, base white-strigose.

Spores black in deposit, 10.7–15.7 × 5.3–7.6 μ, averages 12.45–13.8 × 6.1–6.9 μ, narrow $\frac{D}{d} = 1.9–2.1$, flattened or not, $d_1–d_2 = 0.0–0.6$ μ, narrowly subovate to elliptic in face view, with a more or less distinct suprahilar depression in side view, blackish brown, germpore always very much visible on the abaxial side; basidia tetramorphic, four-spored, 18–30 × 8–10 μ; paraphyses hyaline, inflated; pleurocystidia none; cheilocystidia vesiculose, 20–30 μ broad, ventricose-fusoid ones rare to absent; gill trama thin and appearing cellular in sections, slightly to distinctly vinaceous brown in KOH; pileus trama thin and appearing to be a mixture of narrow filaments and enlarged cells, vinaceous fuscous in KOH, clamp connections present; cuticle of vesiculose cells; pilocystidia subcylindric, rarely much inflated below, apex obtuse, 53–120 (–150) × 8–20(–25) × 4–12.5 μ, somewhat variable in average size in different specimens; sclerocystidia not seen.

Amphithallic, tetrapolar, some specimens simply heterothallic tetrapolar, but the two types interfertile (Lange **1952**), mycelium with numerous clamp connections.

Habit, habitat and distribution: Gregarious or solitary, in moist leaves or on wet black muck, U.S.A.* and Denmark *.

Observations: It has not been possible to find any morphological differences distinctive for the purely heterothallic strains. The species is closely related to *C. plagioporus*, but easily distinguishable by the pilocystidia and the richer purplish colors.

15. **Coprinus sclerocystidiosus** sp. nov.

Illustrations: Lange 1952, Figs. 4, 13.

Statura speciei praecedenti similis, pilei tamen diametro saepe minore, pallide cinnamomeus vel argillaceus.

Sporae fuscae vel pallide fuscae, magn. 10.5–15 × 5.3–7.5 μ, anguste ovali-ellipticae, poris excentricis ornatae; basidia tetramorpha; cheilocystidia globularia, diam. 13–20 μ; pilocystidia dimorpha, altera hyalina, magn. 42–100 × 7.5–15 × 3–7.5 μ, subobtusa, altera sclerocystidia incrustata, magn. 43–166 × 1.5–4.5 μ, acuta.

Fibulae praesentes. Heterothallus, tetrapolaris.

Solitarius vel. subfasciculatim ad terram silvaticam.

Typus 10.8.49 ad Lundeborg, Danmark sub numero 3073 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.6–1.2 cm high, long remaining narrowly conic, then expanding and finally revolute, fisso-sulcate, faintly transversely rugulose in the grooves, pubescent from collapsing cystidia, pale to moderately dark brown, disc "ochraceous salmon" to "ochraceous tawny" or pale "cinnamon brown," striate part paler, soon darkening from the spores, disc remaining brown; flesh thin and fragile, deliquescing in humid weather; lamellulae close to subdistant, 34 reach the stipe, rather narrow, edge white; stipe 2–3 × 0.15–0.2 cm, white, faintly darkened at base which is somewhat strigose.

Spores 10.5–15 × 5.3–7.5 μ, averages (11.2–)12.35–13.5 × (6.2–)6.45–6.85 μ, narrowly ovate in face view, elliptic in side view, $\frac{D}{d} = 1.8–2.05$, slightly flattened, $d_1–d_2 = 0.2–0.5\ \mu$, color relatively pale fuscous in water mounts, in KOH not much darker, germpore large and usually almost fully visible on abaxial side; basidia 20–33 × 9–10 μ, four-spored, tetramorphic, paraphyses subglobose, 13–20 μ broad; pleurocystidia absent; cheilocystidia balloon-shaped, 13–46 μ broad (or in other specimens than the type mostly fusoid-ventricose and 27–35 × 9–13 μ); pileus trama dull brownish, clamp connections seen on thin hyphae; cuticle of hyaline, subglobose cells, mostly 25 μ broad, sometimes

larger, more oblong in the furrows (80–90 μ long); pilocystidia very variable, hyaline ones 42–100 × 7.5–15 × 3–7.5 μ, bottle-shaped, neck narrowly cylindric, apex obtuse; sclerocystidia 43–166 × 5–13 × 1.5–4.5 μ, thick-walled, subhyaline to yellowish brown, slightly inflated below, pedicellate, tapering to a pointed apex, walls often somewhat irregularly wavy, in some specimens incrusted with hyaline, refractive material at the base, the two types of pilocystidia somewhat intergrading, in some specimens only sclerocystidia are seen, on other pilei they are rare.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Scattered to gregarious or even subfasciculate, on wet, rich soil with scattered vegetation, along drives in woods, sometimes attached to small bids of wood, Denmark *, U.S.A. Type: Purreskov, Lundeborg, Denmark, 10-VIII-1949. M. Lange coll. *3073*, in the Univ. of Copenhagen Botanical Museum.

Observations: The description is drawn up on several Danish collections found to be interfertile in culture. We have two American collections from Idaho which possibly may belong here. They differ in having the cheilocystidia fusoid-ventricose and in addition the sclerocystidia differ slightly. These have not been studied in culture and their identification is considered tentative. When the sclerocystidia are abundant, as in most of the Danish collections, they characterize the species well.

16. **Coprinus callinus** sp. nov.

Illustrations: Lange 1952, Figs. 5, 14.

Statura speciei praecedentis similis, colore saturato, cinnamomeobadis; tarde deliquescens.

Sporae magn. 9.7–13.3 × 5.6 μ, subangustae, ovato-ellipticae, fusco-nigrescentes, poris subapicali-excentricis ornatae; basidia tetramorpha, magn. 23–35 × 7.5–9 μ; pleurocystidia nulla; cheilocystidia globularia, diam. 20–30 μ, nonnulla subfusiformia; pilocystidia acuminato-subobtusa, magn. 75–154 × 10–20 × 3.5–10 μ; sclerocystidia plus minus frequentia, illis similia.

Fibulae praesentes. Heterothallus, tetrapolaris.

In callibus silvaticis terricolus.

Typus 30.6.1950 ad Tiselholt, Danmark sub numero 3327 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.5–1.7 cm high, narrowly conic when in bud, expanding but the edge hardly recurving, fisso-sulcate to the disc which is dark brown ("tawny olive" to dark "cinnamon brown" or even

pale "sepia") paler towards the margin; lamellae crowded, becoming subdistant, 30–40 reach the stipe, 1–2 tiers of lamellulae, rather narrow, broadest near the middle, narrowly adnate to almost free, pallid, blackening from the ripening spores, edge white; stipe 2.5 × 0.15 cm, sometimes longer, caulocystidia numerous, white, hyaline in smallest carpophores, base white or faintly brownish; lamellae tardily deliquescing, pileus deliquescing in very humid atmosphere only.

Spores 9.7–13.3 × 5.0–6.0 μ, averages 10.7–11.95 × 5.57–6.2 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.75–2.0$, flattened slightly or terete $d_1–d_2 = 0.1–0.5$ μ, narrowly ovate-elliptic in face view, narrowly elliptic in side view, dark fuscous, germpore subapical to distinctly eccentric; basidia 23–35 × 7.5–9 μ, four-spored, tetramorphic; pleurocystidia absent; cheilocystidia subglobose to globose, 20–30 μ broad, generally with some few bottle-shaped cystidia intermingled (these similar to pilocystidia but shorter and more obtuse); gill trama of somewhat inflated, rather short elements, subhyaline; pileus trama pallid to vivid brownish; cuticle of subglobose cells, 20–25 μ broad, some more oblong seen in the grooves; pilocystidia long, slightly inflated below, acuminate, apex pointed to subobtuse, 75–154 × 10–20 × 3.5–10 μ, almost constantly mixed with a larger or smaller number of thick-walled brownish sclerocystidia similar to the other pilocystidia but slightly shorter, more pointed and brownish.

Heterothallic, tetrapolar, clamp connections seen on mycelium.

Habit, habitat and distribution: In moist places on the ground under sparse vegetation, more rarely on buried twigs and the like, solitary, gregarious or subfasciculate. Most collections are from along drives in the woods, Denmark *, U.S.A. Type: Tiselholt, Denmark, 30–VI–1950, in beech leaves, M. Lange coll. *3227*, in the Univ. of Copenhagen Botanical Museum.

Observations: The above description is based on several Danish collections found to be interfertile in culture. Several other Danish collections, forming a different interfertility group, can, however, not be distinguished on any other character. We have not cultured American material, but many American collections conform well with the above description and should be regarded as belonging in the same species, even if some deviate in minor characters such as slightly larger spores and more obtuse cystidia. The long, subacute pilocystidia and medium sized rather narrow

spores appear to be distinctive of *C. callinus*. Further studies, however, may show the necessity of recognizing taxa below the species level for some of the material we have encountered.

It is highly possible that this is *C. hiascens* in the sense of Romagnesi (**1941**), and it might also be *C. congregatus* of some authors and *C. sociatus* of other writers. The two first mentioned names are here used for other species, and the third name should presumably be discarded since the existing descriptions are very inadequate.

17. **Coprinus subimpatiens** sp. nov.

Illustrations: Lange 1952, Figs. 5, 14.

Statura et colore *C. callino* et *C. sclerocystidioso* valde affinis, sed plerumque major, sulcis latioribus, rugulis transversalibus manifestioribus ornatis.

Sporae magn. 9.3–13.5 × 5.7–7.1 μ, latae, poris subapicalibus-subexcentricis ornatae; pleurocystidia sacciformia, sparsa; cheilocystidia vesicularia, nonnulla ventricosa; pilocystidia magn. 62–110 × 9–17 × 5.5–8.5 μ, hyalina, fusiformi-ventricosa, apice subobtusa; sclerocystidia minora, saepe crebra.

Fibulae praesentes. Heterothallus, tetrapolaris.

In silvis terricola, subfasciicularis vel gregarius.

Typus 22.8.1948 ad Ithaca, Michigan, U.S.A. sub numero 6273 a V. Potter lectus, in Herb. Univ. Mich. depositus.

Pileus 1.5–2.3 cm high, cylindric to ellipsoid at first, becoming obtusely conic with a flaring margin, pruinose when young, but soon naked, plicate-striate to the sordid "tawny" to "cinnamon brown" disc, paler "ochraceous tawny" to "ochraceous buff" or even whitish toward the margin, marginal area soon tinged lead-color (at least between the brownish ridges), transversely rugulose in the grooves, sometimes splitting; flesh very soft and thin, no odor or taste; lamellae close, about 28 reach the stipe, narrow, broadest above, narrowly adnate, white, becoming black, but tardily deliquescing, often crisped, edges long remaining whitish; stipe 2.5–10 × 0.15–0.3 cm thicker in young buds, equal, hollow, covered with caulocystidia, white to the base, loosely fibrillose at base, more appressed fibrillose above, glabrescent in age.

Spores black in deposit, 9.3–13.5 × 5.7–7.1 μ, broad $\frac{D}{d} = 1.6$–1.8, slightly flattened, d_1–$d_2 = 0.25$ μ, dark brown to almost black, coal black in KOH, germpore subcentral to distinctly eccentric; basidia tetramorphic, 18–32 × 8–10 μ; pleurocystidia scattered

(absent in some specimens), vesiculose to somewhat elongated with broadly rounded apices, 26–38 μ broad; cheilocystidia mostly vesiculose, up to 50 μ broad, bottle-shaped cystidia intermingled, the latter 85 × 18 × 7 μ, or smaller (mostly 25–40 × 10–13 × 5 μ); gill trama pale tawny in KOH near flesh of cap, hyaline elsewhere; pileus trama tawny in KOH, cuticle of subglobose cells, 25–35 μ broad, larger and more oblong in the grooves; pilocystidia 62–110 × 9–17 × 5.5–8.5 μ, hyaline, slightly brownish near the ventricose base, neck subcylindric, apex obtuse; sclerocystidia rare to abundant, some of them short and obtuse, others long, wavy (like thick-walled hyphae), dark brown.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habitat, habit and distribution: On mull or on more sandy soil, solitary, gregarious or subfasciculate, U.S.A.*, Denmark*, England*, Sweden*. Type: Ithaca, Mich., U.S.A., 8-22-48, leg. V. Potter (coll. *6273*), in Univ. of Michigan Herbarium.

Observations: The description covers American and Danish material found interfertile in culture and very uniform in morphological characters. Other Danish and Swedish collections and an English specimen have the same morphology, but form two other intersterility groups. They should be included in this species also. This species, which apparently has not been described previously, is close to *C. callinus* and also to *C. eurysporus*. It is distinguished on its broadly ovate spores and the medium sized obtuse cystidia.

Stirps *Eurysporus*

18. **Coprinus eurysporus** sp. nov.

Statura speciei praecedenti similis.

Sporae latiores, magn. 9–10.3 × 6.1–7.8 μ, rotundati-ovali-subglobulares; pleurocystidia fusiformi-ventricosa, sparsa, magn. 36–48 × 9.13 μ; cheilocystidia similia vel vesicularia; pilocystidia magn. 40–70 × 9–14 × 2.5–3.5 μ, acuminata, multa eorum sclerocystidia.

Humi in virgulis.

Typus 21.10.1947 ad Beaver Creek, Mt. Hood National Forest, sub numero *28033* ab A. H. Smith lectus, in Herb. Univ. Mich. depositus.

Pileus 1–2.5(–3) cm broad, subcylindric when young, becoming obtusely conic to convex, very finely pubescent at first, translucent-striate and finally becoming plicate-striate to the smooth disc, margin revolute when deliquescing, color "buckthorn brown"

to near "cinnamon brown," paler toward the margin, margin becoming gray; flesh very thin and delicate, no odor; lamellae crowded, narrow, adnate to stipe apex, edges pallid and crenulate at first, faces becoming dark over all before deliquescence begins; stipe 5–8(–9) × 0.25–0.5 cm, slightly enlarged downward, hollow, white, very fragile, splitting, minutely pruinose at first but soon glabrescent.

Spores 9–10.3 × 6.1–7.8 μ, compressed, d_1–d_2 = 1.0 μ, broadly wedge-shaped with rounded corners or almost circular in face view, in side view obscurely inequilateral to subelliptic, pore broad (2.5 μ), subapical, walls dark chocolate color to almost black in KOH; basidia four-spored, ventricose in midportion when sporulating, tetramorphic, 18–42 × 8–10 μ; pleurocystidia scattered, 36–48 × 9–13 μ, fusoid-ventricose, hyaline, thin-walled, with narrow necks and acute apices; cheilocystidia a mixture of vesiculose cells and those resembling pleurocystidia; pileus trama tawny revived in KOH, cuticle of clavate to subglobose cells; pilocystidia scattered, fusoid-ventricose, hyaline, thin-walled, 40–70 × 9–14 × 2.5–3.5 μ, apices acute, some sclerocystidia also seen, similar to pilocystidia but thick-walled and brown.

Habit, habitat and distribution: Gregarious on sticks lying on the ground, Beaver Creek, Bear Springs Area, Mt. Hood National Forest, Oct. 21, 1947, Smith n. *28033* (type), in the Univ. of Mich. Herbarium.

Observations: The species is established on American material which was not obtained in culture. It is readily recognized by the acute, small cystidia and the short, broad spores. We have a single collection from Denmark which probably belongs here (Lange, coll. *3313*), but this differs in having sclerocystidia exclusively, and in slightly larger spores. Like other species in the group, it is tetrapolar, with clamp connections on the mycelium.

19. **Coprinus amphithallus** sp. nov.

Statura speciei praecedenti similior, sed minor.

Sporae magn. 11.2–15.6(–17) × 6.2–8.3 μ; basidia bispora, tetramorpha, magn. 24–40 × 9–10 μ; pleurocystidia nulla; cheilocystidia fusiformi-ventricosa, magn. 30–50 × 9–18 μ; pilocystidia 40–85 × 9–17 × 5–5.5 μ, ventricosa, apice obtusi-subacuta, hyalina, basi fuscidula.

Fibulae praesentes. Amphithallus.

In silvis terricolus.

Typus 30.6.1950 ad Tiselholt, Denmark sub numero 3222 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.6–1.2 cm high, obtusely conic, expanding to campanulate, the margin revolute as deliquescence progresses, densely cystidiose at first, paler or darker brown "cinnamon brown," "Saccardo's umber" or "ochraceous tawny" over the disc and backs of striae, pallid or even whitish between striae, darkening as spores mature; lamellae narrow, close becoming subdistant, about 16 reach the stipe, adnate, pallid, becoming black before tardily deliquescing; stipe 3–5 × 0.08–0.1 cm, hyaline whitish, sometimes slightly brownish below, pruinose at first, soon glabrous.

Spores varying much in size on same cap, 11.2–15.6(–17) × 6.2–8.3 μ terete or somewhat flattened, d_1–d_2 = 0.6 μ, elliptic in side view, elliptic-ovate in face view, the larger ones often narrowed near apex so as to appear obscurely snoutlike, dark chocolate brown, germpore subapical to distinctly eccentric; basidia all two-spored, tetramorphic, 24–40 × 9–10 μ; paraphyses not distinctive; pleurocystidia absent; cheilocystidia fusoid-ventricose, small, 30–50 × 9–18 μ, apices subacute hyaline and thin-walled; pileus trama pale ochraceous in KOH, cuticle of vesiculose cells, pale yellowish or darker brownish (corresponding to color of cap), pileus trama ochraceous tawny, or darker; pilocystidia 40–85 × 9–17 × 5–5.5 μ, hyaline, except for the tawny to yellowish pedicels, ventricose, apex obtuse to subacute.

Amphithallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: On black mull or much in moist places, Denmark *, U.S.A. Type: Tiselholt, Denmark, June 30, 1950, M. Lange coll. *3222*, in the Univ. of Copenhagen Bot. Museum.

Observations: Only the Danish strain has been brought into culture, but the Danish and American material correspond in all morphological details except for slight differences in color. The species is recognized by the small pilocystidia with thickened basal walls. No four-spored *Coprinus* appears to be closely related.

Stirps *Hiascens*

20. COPRINUS HIASCENS (Bull. ex Fr.) Fr. sensu J. Lange.

Pileus 1.0–2.5 cm high, narrowly glandiform, then broadly convex, expanding rather late and hardly recurving, fisso-sulcate, transversally grooved, pallid, disc darker (pale "cinnamon brown"); flesh thin, without odor and taste, slowly but distinctly deliquescing in humid atmosphere; lamellae moderately crowded to subdistant, adnate to almost free, narrow, pallid, then almost

black from the spores; stipe 4–5 × 0.15–0.3 cm, hollow, white to the base, finely striate above, in young specimens with a few scattered fibrils, densely covered with caulocystidia, not very brittle, slightly enlarged below, hollow.

Spores 7.9–12.1 × 4.5–5.8 μ, averages 9.65–10.9 × 4.9–5.25 μ, hardly flattened, narrow, $\frac{D}{d} = 1.9–2.55$, narrowly ovate in face view, somewhat oblique in side view, apiculus very prominent, germpore large, almost apical, not visible in face view, brown; basidia four-spored, tetramorphic, very different in shape, the long ones much protruding and 36 × 6.5–7 μ, short ones 18 × 8.5–9 μ; paraphyses subglobose, pleurocystidia absent, cheilocystidia numerous, bottle-shaped, subacuminate, 26–75 × 12–18 × 4–7 μ; trama of gills scanty, of rather narrow and a few inflated elements; pileus trama of rather coarse elements, very pale cinnamon, cuticle of subglobose cells about 20 μ broad, longer in the grooves, pilocystidia 80–175 × 11.5–25 × 4–9 μ, very long, more or less inflated below, hyaline, some of them slightly brownish at base, no sclerocystidia seen.

Heterothallic, tetrapolar, clamp connections seen on mycelium.

Habit, habitat and distribution: In small clusters, rarely solitary, found both on rich and very light soil, Denmark.

Observations: The species is well characterized by the size and shape of the spores, resembling those of *C. disseminatus*, with which species it also has some likeness in basidial characters. In addition, the pilocystidia are quite characteristic. This is undoubtedly the species depicted by J. Lange (**1939**), and probably also the one described by Ricken (**1915**), except for spore measurements, which Ricken quotes from Karsten who presumably used the name for *C. plicatilis* or a species closely related to it. Romagnesi's species (**1941**) is presumably *C. callinus*.

Stirps *Disseminatus*[3]

21. COPRINUS DISSEMINATUS (Pers. ex Fr.) S. F. Gray (1821).

This species is so frequently described that a repetition of the macroscopic morphology is superfluous here (cfr. Buller **1913**,

[3] The American author is not in agreement with placing these non-deliquescing fungi in *Coprinus*, but they have been retained in this paper in order to put certain data on record. The conclusions recorded under this stirps are those of the Danish author.

1924 and Kuhner **1926**). The following is a summary of the microscopic data.

Spores 7–9.4(–11) × 3.9–5.5 μ, flattened, d_1–d_2 = 0.25–0.6, rather broad $\frac{D}{d}$ = 1.6–1.9, broadly ovate in face view, obliquely elliptic in side view, apiculus rather conspicuous, germpore apical, relatively pale brown; basidia 20–55 × 5–10 μ, four-spored, distinctly tetramorphic, long type much protruding, very narrow; pleurocystidia none, cheilocystidia developed from the cap margin and about half way up the gill edges, rather scattered, large, obtuse, 60–115 × 16–21 × 7.5–10 μ; pilocystidia very large, 76–215 × 19–29 × 8–12.5 μ, inflated below, sometimes incrusted at the base, apex obtuse; sphaerocysts rather numerous, incrusted, 10–40 μ broad.

Heterothallic, bipolar, clamp connections present on mycelium.

Habit, habitat and distribution: Densely cespitose to gregarious, rarely subsolitary, on and around stumps. Almost cosmopolitan; we have seen material from several European countries, from U.S.A. and from India.

Observations: In growth form and stature and especially in the microscopic characters, this species is clearly a very close relative of *C. silvaticus*. There is a great likeness in the way the different types of cystidia are developed and in the rather outstanding type of basidia, also the spore shape is—in spite of differences in size and configuration—basically the same, and finally the type of sphaerocyst is identical. The two species are probably not very intimately related to the members of the subsection *Setulosi*, but more to the other fasciculate, semilignicolous species around *C. disseminatus*. This will leave no possibility for retaining the genus *Pseudocoprinus*.

22. **Coprinus subdisseminatus** M. Lange, sp. nov.

Pileus 0.3–0.8 cm altus, late convexus, deinde convexo-planus, sed non revolutus, distanter sulcatus, pallide cinnamomeus, velo debili, fibrillari primo indusiatus; stipes hyalino-albidus, disculo myceliari exiguo, membranaceo basi cinctus, non vel tardissime deliquescens.

Sporae magn. 8.8–12.7 × 5.5–6.5 μ, pallide fuscae; basidia plerumque trimorpha, tetraspora, magn. 19–33 × 9–10.5 μ; pleurocystidia nulla; cheilocystidia sacciformi-lageniformia, magn. 30–75 × 11–20 × 7–12 μ; pilocystidia magn. 59–121 × 10–22 × 7.5–15 μ, hyalina, subcylindrica, apice obtusa.

Fibulae praesentes.

In silvis humum uvidum et quisquilias inhabitans.

Typus 20.6.1950 ad Elsehoved Denmark sub numero 3216 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.3–0.8 cm high, rather broadly convex in the bud, convex-flat when expanded, but not revolute, sulcate to the disc, the creases rather distant, more or less transversally grooved, pale brownish, "cinnamon buff" to "cinnamon," cystidiose; flesh very thin and fragile, pale, not deliquescing, odor and taste not distinctive; lamellae subdistant, 16–24 reach the stipe, adnate or slightly decurrent, pallid when young, dark "cinnamon brown" when spores ripen; stipe 1.3–2.5 × 0.05–0.15 cm, hyaline whitish to the base, slightly fibrillose, pubescent, on some very young specimens the fibrils from a rudimentary veil, attached to the substratum by a more or less developed whitish mycelial disc.

Spores 8.8–12.7 × 5.0–6.5 μ, averages 10.35–11.55 × 5.4–5.9 μ, mostly rather narrow, $\frac{D}{d} = 1.8–2.05$, germpore subapical to somewhat excentric, slightly visible on abaxial side on some spores, pale chocolate brown; basidia four-spored, mostly trimorphic, 19–33 × 9–10.5 μ pleurocystidia absent; cheilocystidia rather numerous to scattered, broad, obtuse, sack-shaped to bottle-shaped, 30–75 × 11–20 × 7–12 μ; trama of pileus and gills pale brownish, of moderately inflated to rather thin elements, cuticle of subglobose cells, 25–50 μ broad, larger and more oblong in the furrows, pilocystidia 59–121 × 10–22 × 7.5–15 μ, numerous to scattered, subcylindric, not much inflated below, apex obtuse, hyaline or almost so, no sclerocystidia seen.

Heterothallic, tetrapolar, clamp connections seen on the mycelium.

Habit, habitat and distribution: On wet, black mull or on sticks and other vegetable matter in humid localities, solitary or in small groups, but never seen subfasciculate; Denmark *. Type: Elsehoved, Denmark, June 20, 1950, M. Lange coll. *3216*, in the Univ. of Copenhagen Botanical Museum.

Observations: The species is distinguished primarily by its macroscopic characters, namely, the small, broad, pallid pileus. It is also well characterized by its cystidia. It has the appearance of *C. disseminatus* but its growth form is different, and the microscopical features are clearly distinct. We have an American collection, from Michigan, which may represent this species, but we have noted the cheilocystidia to be vesiculose.

23. COPRINUS IMPATIENS (Fr.) Quélet.

Pileus 1.5–3 cm high, broadly ovate at first, expanding to campanulate but edge not recurving, deeply grooved, even before opening, by alternating long and short impressions from the edge and about three quarters in, minutely cystidiose, pallid alutaceous, the disc slightly darker, fading to whitish when drying, becoming lead gray with a brownish tinge when spores mature, flesh thin and fragile, not deliquescing, lamellae subdistant, becoming fuscous when spores ripen; stipe 5–10 × 0.1–0.4 cm, somewhat enlarged towards the base which is white strigose, hollow, whitish, pubescent from cystidia.

Spores 8.5–11.1 × 5–6.1 μ, almost terete d_1–d_2 = 0.15–0.3 μ, rather broad, $\frac{D}{d}$ = 1.75–1.85, broadly ovate in face view, broadly elliptic in side view, pale chocolate color, germpore almost apical; basidia four-spored, pleurocystidia absent, cheilocystidia numerous, bottle-shaped to flask-shaped, generally subacute to acute, 20–62 × 10–16.5 × 3.5–5 μ; pilocystidia rather few, narrowly conical, acute to subacute, 50–86 × 10–16 × 3–4.5 μ, hyaline or slightly brownish below.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Solitary, rarely subfasciculate, in deep foliage, Denmark *, France *, and the United States.

Observations: The species is one of the largest in the group, quite well distinguished by the broad grooves on the cap, the small, comparatively pale spores and the cystidia. *C. digitalis* in the concept of Romagnesi (**1941**) might be this species, but *C. digitalis* distributed by Lundell and Nannfeldt (No. *101*) most likely is a closely related but distinct species, recognized macroscopically by its more brownish gills, and microscopically by still smaller spores (7.85 × 4.7 μ) and also by the presence of some globose cheilocystidia between the bottle-shaped ones. The cultures deposited in Baarn under this name by Yen have been found interfertile with our material. The two last mentioned species are non-deliquescing, at least under ordinary conditions. Hence they belong in the genus *Pseudocoprinus* as generally defined at present. The American author would prefer to maintain this division while the European author finds the species too closely related to

members of the subsection Setulosi to make a generic separation possible (cp. Lange **1952**).

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BITANCOURTIA CASSYTHAE ON CASSYTHAE FILIFORMIS AND PROPOSED NOMENCLATORIAL CHANGES AMONG OTHER MYRIANGIALES[1]

M. J. Thirumalachar and Anna E. Jenkins[2]

(with 2 figures)

Near Bangalore, India, many plants of *Cassytha filiformis* are parasitized by a myriangiaceous fungus. The small dark stromata are abundant over the entire stem. Regeneration of new shoots may be prevented or plants killed outright. The ascomycete seems not to be characterized by any genera of the Myriangiales so far described.

Bitancourtia[3] gen. nov.

Stromata ascigera in pustulis conidiiferis mox evoluta, superficialia vel intra-epidermalia, erumpenti-superficialia, pulvinata vel pulvinato-applanata, sessilia, basi lata, tota matrici affixa; epithecium atro-fuligineum, ex cuticula et epidermidis parietibus superis ruptis et pseudoparenchymate obscuro formatum, friabile, particulis in superficiebus stromatis ascigeri persistentibus vel oram conspicuam interdum ruptam formantibus; stroma ascigerum brunnescens, griseolum, vel viridulum, et pulveraceum, intus prosenchymaticum, brunneo-flavidum, loculis numerosis, in stratis dispersis, ascum unicum ferentibus; hypothecium pseudoparenchymaticum, tenue, obscurum; asci globosi vel ellipsoidei; ascosporae elongato-oboyoideae vel fusiformi-ellipticae, transverse 3-septatae, hyalinae; stromata conidiifera subcuticularia vel intra-epidermalia, palis conidiophororum obscuris; conidia minuta, 1-cellularia, hyalina vel lutescentia; microconidia adsunt.

Type: *Bitancourtia cassythae*.

[1] This study is a contribution under the Point 4 program, administered by the Technical Cooperation Administration, U. S. Department of State.

[2] Dr. Thirumalachar, Government Penicillin Factory, Poona, India; Dr. Jenkins, Service Foreign Agricultural Relations, U. S. Department of Agriculture, formerly of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

[3] The name *Bitancourtia* is proposed in honor of A. A. Bitancourt, whose contributions to the study of the group have greatly enriched our knowledge.

Bitancourtia cassythae sp. nov. FIG. 1, A–G; 2

Maculae minutae, atrae nitidae, in caulibus omnibus dispersae, numerosae; hyphae inter- vel intra-cellulares, septatae, brunneo-flavidae; stromata ascigera in pustulis conidiiferis mox evoluta, solitaria, gregaria, vel coalescentia, superficialia, vel intra-epidermalia, dein erumpenti-superficialia, pulvinata vel pulvinato-applanata, sessilia basi lata tota matrici affixa, 450–760 μ diam. $\times$ 130–160 μ crassa; epithecium atro-fuligineum, ex cuticula et epidermidis parietibus superis ruptis et pseudoparenchymate obscura formatum, usque ad 25 μ crassum, friabile, particulis in superficiebus stromatis ascigeris persistentibus vel oram conspicuam interdum ruptam formantibus; stroma ascigerum brunnescens, griseolum, vel viridulum, et interdum pulveraceum, intus prosenchymaticum brunneo-flavidum, loculis numerosis, in stratis dispersis, ascum unicum ferentibus; hypothecium pseudoparenchymaticum, tenue, obscurum; asci globosi vel ellipsoidei, 8-spori, 22–32 μ $\times$ 11–19 μ; ascosporae elongato-oboyoideae vel fusiformi-ellipticae, interdum asymmetricae vel curvatae, leves, transverse 3-septatae, hyalinae, 14–21 μ $\times$ 4–5 μ; stromata conidiifera subcuticularia vel intra-epidermalia dein erumpentia, interdum ascomata cingentia, obscura, usque 180 μ diam. $\times$ 100 μ crasa; palis conidiophororum compactis; conidiophora obscura, oblonga, cylindrica, vel acuta, 1-pauciseptata, 5–26 μ $\times$ 4–5 μ; conidia ellipsoidea, 1-cellularia, hyalina vel lutescentia, 3 $\times$ 5 μ; microconidia adsunt.[4]

In caulibus *Cassythae filiformis* L. (Lauraceae), Yashavantapur, Bangalore, South India, parasitica.

Yashavantapur, Bangalore, South India, Jan. 1, 1948, M. J. Narasimhan (Type). Specimens distributed as follows: Herb. Crypt. India Orient., New Delhi, India.; Herb. Royal Bot. Gard., Kew, England; No. *90598*, Mycol. Coll., U. S. Bur. of Plant Indus., Soils, and Agr. Engin., Beltsville, Md., No. *5319*, Herb. Secc. Fitop. Inst. Biol., São Paulo, Brazil, and No. *488*, Jenkins-Bitancourt, Myriangiales selecti exsiccati.

A large collection of *Cassytha filiformis*, Marshall Islands, 1951, F. R. Fosberg, was lent to us by the collector to examine for the possible presence of *Bitancourtia cassythae*. None was found.

In comparing the fungus on *Cassytha* with several myriangiaceous fungi previously described, *Myriangium pritzelianum* P. Henn. (**2**, p. 353) proved to be of interest. The fungus parasitizes leaves, stems and flowers of *Trymalium wichurae* Nees. (Rhamnaceae) in Australia, and the presence of numerous dark, pulvinate stromata especially on the stems suggested a similarity

[4] Thanks are expressed to Conrad V. Morton for translating the diagnoses of the new genus and species into Latin.

to *Bitancourtia cassythae*. A careful examination of the stromata, however, revealed that they are cinnamon color as described. This coloration is characteristic of *Uleomyces*. Comparison of the distribution of the asci with those of other species of *Myriangium* and of *Uleomyces* indicates that the fungus is more closely related to *Uleomyces* than *Myriangium*. We therefore propose to transfer it to *Uleomyces* as **U. pritzelianum** (P. Henn.) comb. nov. (FIG. 1, H, I).

The fungi concerned in the remainder of this paper were all discovered in South America.

The genus *Kusanoopsis* Stevens & Weedon (**8**, p. 199) was established for a myriangiaceous fungus on an undetermined dicotyledonous plant in British Guiana. The genus is so far monotypic. An examination of authentic material of the type species, *K. guianensis*, together with microtome sections prepared by Stevens and Weedon[5] and freehand sections made by the senior writer, demonstrates that *Kusanoopsis* is synonymous with *Uleomyces* P. Henn. (**1**, p. 107). The Lauraceae are known to be hosts for species of *Uleomyces*. A general comparison of the "unknown dicotyledonous host" of *K. guianensis* with herbarium specimens of Lauraceae shows that this may well belong to the same family.

It is proposed to reduce *Kusanoopsis* as a synonym of *Uleomyces* with the following synonomy:

Uleomyces guianensis (Stevens & Weedon) comb. nov.

Kusanoopsis guianensis Stevens & Weedon (1923)

On ? Lauraceae

Stevens and Weedon (**8**, p 201) described *Myriangina miconae* as inciting a small leaf spot on *Miconia* sp. in British Guiana. This fungus as described and illustrated by them is clearly of the genus *Elsinoë* Rac. emend. Jenkins & Bitanc. (**4**). Not only has this been noted by the writers, but it has been mentioned independently by both D. P. Limber and A. A. Bitancourt in correspondence with the junior writer. A reexamination of Stevens

[5] Stevens and Weedon's microtome slides mentioned in this paper are deposited with their specimens in the herbarium of the University of Illinois. They were kindly lent to the junior writer by Dr. Leland Shanor.

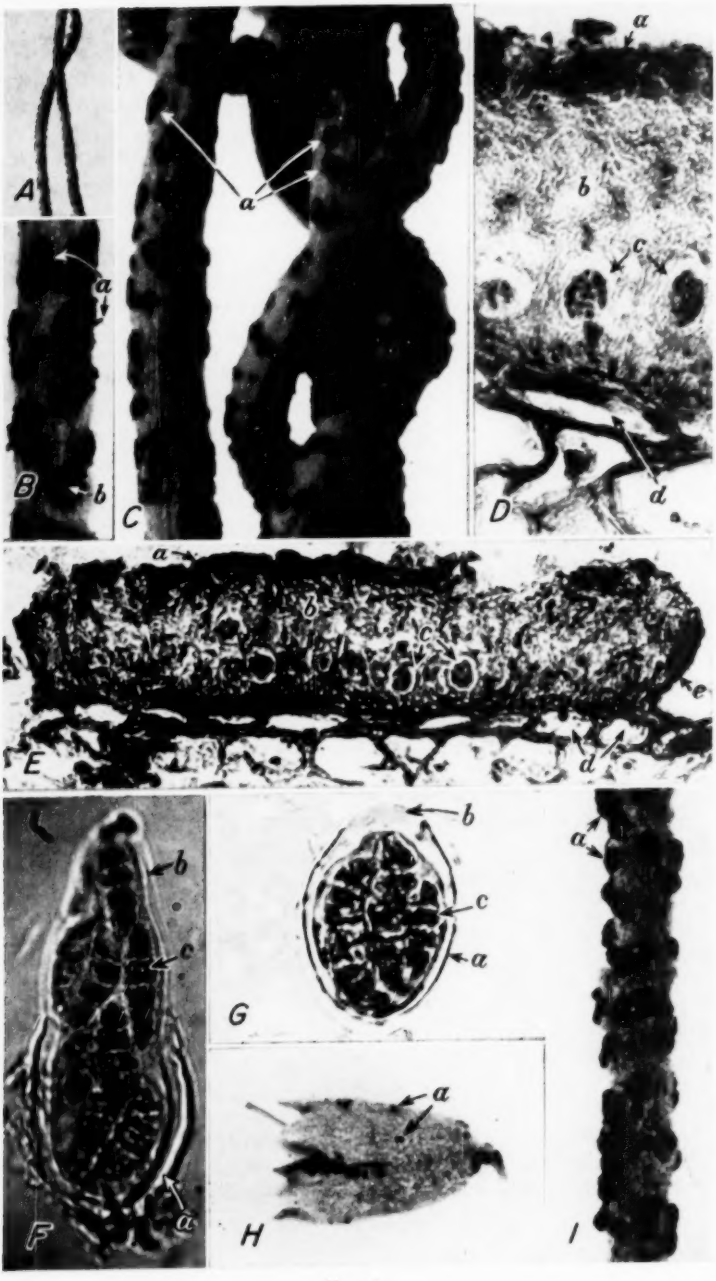

FIG. 1.

and Weedon's microtome section of this species also supports this conclusion. The following nomenclatorial change is proposed:

Elsinoë miconiae (Stevens & Weedon) comb. nov. Limber & Bitanc.

Myriangina miconiae Stevens & Weedon (1923)

The ascomycete on *Mimosa vellozina* described by Rehm (**5**, p. 169) as *Ascomycetella purpurascens* and transferred to *Myriangium* (*Ulcomyces*) by Hennings (**3**, p. 56) was recognized more recently by Viégas (**10**, p. 59) as *Elsinoë purpurascens* (Rehm). On leaves, the fungus is restricted to the veins, forming the characteristic patterns described and illustrated by Viégas. Comparison of part of the type of *Chaconia berroana* Speg. (**6**, p. 29) on *M. flagellaris* sent to the junior writer by J. C. Lindquist with a specimen of *E. purpurascens* contributed previously by Viégas reveals that the two are identical. This myriangiale seems to conform most nearly to *Ulcomyces*. The following combination is proposed:

Uleomyces purpurascens (Rehm) comb. nov.

Ascomycetella purpurascens Rehm (1901)

Myriangium (*Ulcomyces*) *purpurascens* (Rehm) P. Henn. (1902)

Elsinoë purpurascens (Rehm) Viégas (1944)

Chaconia berroana Speg. (1912)

The genus *Whetzeliomyces* Viégas (**11**, p. 561) was erected to classify a myriangiaceous fungus on *Eupatorium* sp. in Brazil. The conidial stage of the fungus was described by Stevens (**7**, p. 371) on *Eupatorium* in British Guiana. In naming his ascomycete from Brazil, Viégas made the new combination *W. niger* (Stevens).

FIG. 1. *Bitancourtia cassythae* on stems of *Cassytha filiformis*. A–C. Ascomata, general appearance A, ×1; B. *a*, ruptured epithecium exposing the ascogenous stroma (*b*) beneath, ×10; C, ×9. D, E. Part of a stroma (D), ×280, and an entire stroma (E), ×160, in cross section. *a*, epithecium; *b*, fertile stroma; *c*, asci with ascospores; *d*, epidermis; E, *e*, darkened adhering cuticle. F, G, asci, *a*, outer inelastic wall, ruptured in F; *b*, inner elastic wall, expanded in F; *c*, ascospores; F, ×1250; G, ×1000. H, I. Stromata of *Uleomyces pritzelianum* on flower (H, *a*), ×6, and stem (I, *a*), ×12, of *Trymalium zeehurae* (type). Photographs by Eugene Herling (A and C), R. L. Taylor (B, H and I) and M. L. Jaeger (D–G).

As restudied by the writers on the basis of the type specimen, this ascomycete is referable to the genus *Agostaea* Theiss. & Syd. (**9**, p. 359). A Latin diagnosis of the species is here provided under the name *A. nigra*.

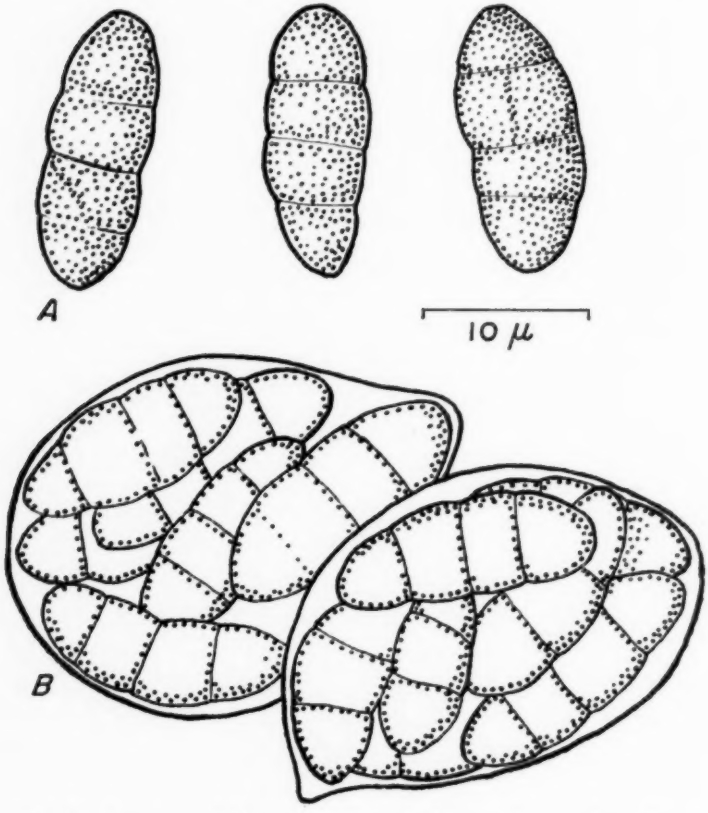

FIG. 2. Ascospores (A) and asci (B) of *Bitancourtia cassythae*. Drawings by D. P. Limber.

Agostaea nigra (Viégas) comb. nov.

Tuberculariа nigra Stevens (1901)

Whetzeliomyces niger (Stevens) Viégas (1945)

Stromata nigra, subepidermalia, erumpentia, hemispherica, lobulata, usque 400 μ in diam., 200 μ crassa, solitaria vel confluentia, e contextu pro-

senchymatico composita, olivaceo-viridia sed rubro-tincta; asci in strato singulo peripherici, e textura intertheci alia separati, subglobosi usque globoso-piriformes, bitunicati, octospori; ascosporae hyalinae sed humidae pigmentum stromatis absorbentes, muriformes, plerumque transverse 3-septatae, cellulis 1–4 verticaliter septatis, ad septa transversa subconstrictae, 11–16 × 5–6 μ.

Status conidicus: *Tuberculariа nigra* Stevens.

In foliis, caulibus et pedunculis *Eupatorii* sp., Brasilia.[9]

Type specimen: Mogi-Mirim, State of São Paulo, Brazil, Dec. 8, 1943, A. S. Lima and A. P. Viégas (No. *4333*, Herb. Inst. Agron., Campinas, Brazil; No. *90130*, U. S. Mycol. Coll.; No. *5395*, Herb. Inst. Biol).

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[9] This translation into Latin was kindly made by Edith K. Cash.

NOTES AND BRIEF ARTICLES

Systemic Infection in Cintractia junci[1]

In discussing the biology of the caricicolous species of *Cintractia*, Savile (Can. Jour. Bot. **30**: 410–435. 1952) recently indicated that none appeared to be systemic, despite statements to the contrary; but that specimens suggested that the species attacking *Juncus* and *Luzula* appeared to be in a different category.

When *Cintractia junci* (Schw.) Trel. was found on *Juncus dudleyi* Wieg., near Ottawa, on 19 June 1952 (DAOM *28627*, Savile and Parmelee *3103*), this question was borne in mind. It was noted that culms were never lightly affected but were always completely blasted or completely normal. Infected culms were, on the average, shorter than normal culms and the inflorescence generally had abnormally short branches. It was further noted that infected culms tended to occur in groups, rather than being randomly scattered; and all infected culms were in an area about 3 feet in diameter, although the colony extended for several yards. A number of clumps were carefully dug, in order to preserve rhizome connections as completely as possible. One of the resulting clumps is shown in Fig. 1. All the culms arising from one part of the rhizome were healthy, and all those from the other part were infected.

Free-hand sections were made from various parts of infected culms and mounted in lactophenol with cotton blue. Extremely sparse mycelium could be seen in the xylem in the lower parts of the culm; but sections cut one-half inch below the base of the inflorescence showed plentiful mycelium both in and between the xylem elements. Such hyphae are absent from healthy culms. It is accordingly clear that the fungus is systemic and perennial.

It may be noted that the species of *Cintractia* attacking *Fimbristylis* and *Cyperus* appear always to be localized like those on

[1] Contribution No. 1270 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

FIG. 1. *Cintractia junci* on *Juncus dudleyi*, showing infected culms arising from part of rhizome in which mycelium was perennial.

Scirpus, *Kobresia*, *Carex*, and *Uncinia*, although infection is not confined to the ovaries. However, some, at least, of the species that attack *Rhynchospora* seem to be systemic. If all the species

attacking Juncaceae and all those on *Rhynochospora* prove to be systemic, then the type of habit would seem to have taxonomic significance.—D. B. O. SAVILE AND J. A. PARMELEE.

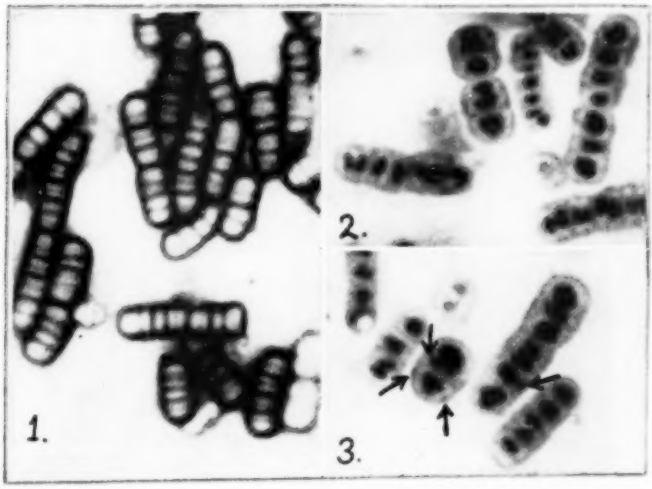
THE CYTOLOGY OF CARYOPHANON LATUM

In two recent papers DeLamater (**1951**, **1952**) has claimed to demonstrate true mitosis in the large bacterium *Caryophanon latum*. These are both illustrated with the same photomicrographs of material acid-hydrolysed, stained with thionin and dehydrated in freezing alcohol. It has been shown that this technique is not, as DeLamater claims, specific for the nuclear structures, but also stains the cytoplasmic septa of the multicellular bacteria which he (apparently unknowingly) employs for his demonstrations, and that, in addition, the method of dehydration produces gross distortion (Bisset, **1953a**). Indeed, in this laboratory it has been observed that exactly similar appearances to those figured by DeLamater can be obtained by the substitution of boiling for freezing reagents in his technique.

An excellent study of *Caryophanon* by Pringsheim and Robinow (**1947**) shows that the vegetative forms are composed of very small, disc-shaped cells, separated by cross-walls, as illustrated here (FIG. 1), and containing typical bacterial nucleoids (FIGS. 2, 3). DeLamater ignores this very striking and characteristic structure, which is clearly shown here in material stained by his method, but, instead of being subjected to his dehydration technique, sealed in water under a coverslip, and photographed immediately.

DeLamater's photomicrographs are, by comparison, very indistinct, and the most characteristic stage, the "metaphase," is represented in both papers by part only of one organism, of which the outline is barely discernible except as two vague marks which presumably represent the "centrioles." Similar stainable areas appear at or near the junctions of cell walls and cross-walls in many bacteria (Bisset, **1952**, **1953a**, **1953b**), and are indicated, in positions exactly corresponding with DeLamater's "centrioles," in FIG. 3. In FIG. 4 a drawing of this "metaphase" is compared

with a typical organism from FIG. 1 and one from FIG. 3, from which it can be seen that the claim to demonstrate a mitotic cycle with this as the sole example, in two papers, of the most characteristic stage, can only be regarded as rash. This, and all De-



FIGS. 1–3. The cytology of *Caryophanon latum*. All photomicrographs of material stained with aqueous dyes, unfixed and mounted in water; photographed immediately after being prepared. 1. Cell walls and cross-walls by phosphomolybdic acid and methyl green, ×2000; the cells are seen to be very small, discoid and numbering as many as six or seven in the very shortest organisms, up to twenty in the largest. 2, 3. Nuclear structures and basophilia of the cell envelopes by HCl-thionin, ×3000; the typical bacterial nucleoids are seen, and the granules at the junction of cell wall and cross-walls, indicated by arrows in 3, correspond to DeLamater's supposed "centrioles."

Lamater's photomicrographs, show appearances which are much more readily interpreted as the somewhat shrunken remains of typical bacterial nucleoids, as figured here and by Pringsheim and Robinow, than as true mitotic figures; especially as DeLamater's interpretation takes no cognizance of the remarkable cellular organization of *Caryophanon,* in which most, if not all, of his "mitotic figures" occupy the space of two, three or more of the relatively tiny, discoid cells of this strongly septate organism.

DeLamater claims also to have demonstrated mitosis in other types of bacteria. The validity of this claim may be examined by those who care to study the present writer's analysis of this evidence, much of which is based upon repeated republications of a single photomicrograph of a "metaphase spindle" (Bisset, **1952**, **1953b**). In every case, including that of micrococci, exactly as in the case of *Caryophanon*, the septate structure and mode of divi-

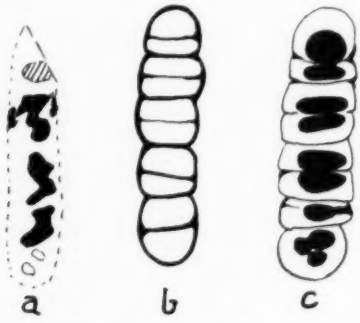


FIG. 4. Drawings comparing DeLamater's "metaphase spindle" (a) with appearance of organisms from FIGS. 1 and 2 in this paper (b and c). His interpretation of this organism, the sole representative of the stage in two papers (DeLamater **1951**, **1952**), is quite incompatible with the complex cellular structure of *Caryophanon*.

sion of these bacteria is ignored by DeLamater, and no evidence is offered in support of his crucial and readily disprovable assertions that his staining method is specific for nuclei and his dehydration technique preserves the structure of the cell unaltered.—K. A. BISSET, University of Birmingham.

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COMMENT ON ABOVE

A complete refutation of K. A. Bisset's contentions concerning this and other studies published by myself will be found in the International Review of Cytology, Volume 2, 1953.—E. D. DELAMATER.

AN UNUSUAL PSILOCYBE

A rather anomalous fungus has developed in a culture of cow dung from Montana which was sent to Dr. Alexander H. Smith. The normal fruiting bodies are those of an undetermined species of *Psilocybe* closely related to *P. coprophila*. The characteristic which attracted special attention to this plant was its production of an aberrant type of fruiting body. The microscopic characteristics of these structures are the same as those found in the normal fructification, but the carpophores are gastroid. The stipe is much reduced or absent and the pileus remains unexpanded. The hymenial layer is dispersed around the periphery of cavities formed by the folded gills. In gross form this type of fruiting body resembles a Gasteromycete more than an agaric. The gastroid fruiting bodies differ from the various types of carpophoroids which have been reported in that they produce viable spores. They have been produced repeatedly in agar culture along with normal fructifications and many intergrading types. Both normal and gastroid fruiting bodies have been obtained in culture from spores and from subcultures of diploid mycelium. No genetic basis for the inheritance of the gastroid form has been established.

While studying the gastroid form in culture, my attention was drawn to another anomalous form which apparently arose as a somatic mutation. This is an albino form which is completely devoid of color and has hyaline spores. It has been grown in culture through six generations from spores and is apparently quite stable. Both normal and gastroid fructifications are pro-

duced by the albino. They have been crossed with the normal form having colored spores and both albino and normal colored forms are obtained in the test crosses. Further experimentation is being carried on and it is hoped that a more detailed report of the behavior of these forms may be presented at some future time. A study of the albino form may be of interest to taxonomists, and particularly to those who have collected white forms whose microscopic characteristics show close affinities with the dark spored species.—KENT H. MCKNIGHT, University of Michigan.

A NEW SPECIES OF AMANITA

Amanita flavivolva sp. nov. Pileo convexo-plano, 6–7 cm lato, subflavo, disco griseo, grato; lamellis adnatis, confertis, 6 mm, albis; sporis 8 × 5–6 μ; stipite albo, flocculoso, 7–9 × 1–1.5 cm; annulo albo, amplo; volva flava, obliterata.

Pileus convex to expanded, scattered, 6–7 cm broad; surface pale-yellow with grayish disk, sparsely decorated with flat volval patches; margin even, entire, whitish; context white, unchanging, 4 mm thick, odorless, mild, becoming acrid; lamellae adnate, crowded, inserted, 6 mm wide, white, unchanging, fringed; spores broadly ovoid, 1-guttulae, about 8 × 5–6 μ; stipe tapering upward, white, unchanging, glabrous and ridged above the veil, flocculose-pulverulent below, 7–9 × 1–1.5 cm; annulus fixed 2 cm from apex, large and skirtlike, white with flavous edge; volva adnate to a small ovoid bulb, flavous, soon breaking into fragments remaining on the ground and cap.

Type collected by W. A. Murrill in an azalea bed under a laurel oak in Gainesville, Fla., July 16, 1950 (*F 19598*). Near *A. gemmata* but having a yellow volva.—W. A. MURRILL.

THE PETERSHAM FORAY

The fourth annual foray (new series) of the Mycological Society of America was held August 18–20, 1949 at the Harvard Forest, Petersham, Massachusetts. The late Dr. W. Lawrence

White, then vice-president of the society, served as foray chairman. Dr. Hugh M. Raup, Director of the Harvard Forest, generously placed most of the facilities of this institution at our disposal. The foray was well attended, with 51 members and friends registering for the event. A few participants stayed in the town of Petersham. However, Shaler Hall, on the Forest grounds, provided living quarters for the majority of our group, and the large laboratory in the same building was made available for demonstrations, discussions, and specimen study.

The foray began at 9:30 A.M. on August 18th. Dr. Raup led the initial field trip and gave an informal lecture-demonstration of the various type localities and experimental plots in the forest. This served as a valuable orientation to the kinds of collecting areas available to the foray. The more avid collectors acquired a large number of specimens on this journey.

This foray became more marked by informal collecting than other forays have been. Throughout the afternoon of the 18th and all of the two following days small groups would vanish from Shaler Hall to visit different parts of the forest. The 2300 acres of the forest contained so many attractive collecting sites that the tendency for the foray to stick together in one large group (as was originally planned) did not persist very long. Each specialist turned to some area he had spotted that most interested him.

Dr. White scheduled a speaker for each evening the foray was in session. This departure from tradition was a striking success. Partially because of this the foray has been regarded by some of its participants as much a "talking foray" as a "collecting" one. The first lecture, presented on the evening of August 18th by Dr. V. M. Cutter, then of Yale, was entitled "*Sporobolomyces* as an organism for genetical and biochemical research." Dr. Cutter showed the group many cultures of *Sporobolomyces salmonicolor* Kluyver and van Niel, first demonstrating the range of variation in isolates from nature, then demonstrating the range of variation he had been able to induce through ultra-violet and x-ray irradiation. The variants selected for illustration were primarily those involving color strains. This material proved ample basis for a lively discussion centering around the following points: (1) The

possibility of using the mutation induction technique to recapitulate the course of phylogenetic evolution in a natural population; (2) The use of induced mutations prepared in the laboratory to predict the range of variation in a natural "species"; (3) The possibility of interpreting the survival values of any given mutation by studying the mutants in competition with the wild types in the field; (4) The possibility of using an organism such as *Sporobolomyces* as a tool for the study of experimental evolution.

Unfortunately the weather preceding the foray had been rather dry. Among the more interesting observations made, Dr. C. R. Orton noted that *Calyptospora columnaris* Kühn on *Vaccinium corymbosum* L. was abundant in one section of the forest and scattered in other areas. In one section where it was most abundant, there were several specimens of *Abies balsamea* (L.) Mill. in close contact with *Calyptospora*. Despite diligent search, in no case could rust in the *Abies* be found. Dr. Orton also reported abundant infection of *Uredinopsis* on *Osmunda* and again he could find no alternate stage on *Abies*. Dr. C. W. Dodge obtained material of *Elaphomyces asperulus* Vitt., a species seldom collected, though this area is not out of its expected range. Five species of myxomycetes and forty-three species of agarics were collected, which is of some interest considering the dry conditions that prevailed during and preceding the foray. Dr. C. J. Alexopoulos and Dr. C. T. Rogerson collected an interesting fungus on spiders, later identified by Dr. E. B. Mains as *Gibellula pleiopus* (Vuill.) Mains (cf. *Mycologia* **42**: 318–319. 1950).

The lecture on Tuesday evening (August 19th), given by Dr. James W. Sinden of Pennsylvania State College, was entitled: "Recent developments in the mushroom growing industry." A large amount of experimental work, done primarily by Dr. Sinden and his colleagues, was described in some detail and the foray members were given a vivid picture of the manner in which some of this research had given rise to technical advances in the industry. Dr. Sinden pointed out that in modern plants the mushroom industry has become almost completely mechanized since the introduction of the tray system in 1934. The compost is turned mechanically and filled into trays mechanically. It is then pas-

ANNUAL FORAY, 1949, HARVARD FOREST, PETERSHAM, MASSACHUSETTS

Left to Right: Top Row: White, Beneke, Fergus, Rogers, Walters, Mrs. Walters, Pady, Bergman, Chable, Howard, Smith, Mrs. Overholts, Miss Haff, Mrs. Dodge, Johnson. Middle Row: Porter, Mrs. Alexopoulos, Miss Weintraub, Mrs. Snell, Conners, Cutter, Snell, Kneebone, Reese, Rogerson, Miss Kuhnen. Botton Row: Alexopoulos, Burlingham, Scott, Sproston, Mrs. Sproston, Dodge, Henry, Orton, Raup.

teurized in a specially insulated and equipped room. After mycelium has started here the trays are covered with an inch of soil mechanically and removed to the growing room where optimum environmental conditions for production of the crop can be maintained. Dr. Sinden described the development of a synthetic compost which competes with horse manure in price, but this has not been widely adopted because of the necessity of obtaining and combining the necessary ingredients. Composting practice has been changed to reduce the great loss in dry weight formerly experienced, resulting in considerable conservation of the ever dwindling supply of horse manure. Spawn for use in planting the beds is now largely made on grain, as this medium, being granular, is easier to handle than the solid manure spawn formerly sold. It is easier to make and also provides a richer medium, giving the mushroom a faster start in the bed.

Your foray chairman requested the writer to prepare this report for the society. The report was prepared but its submission has been delayed because for some time no adequate group picture of the foray could be located for reproduction with the report. These difficulties were recently solved by Mr. Maurice B. Walters, who kindly provided the negative from which the accompanying figure has been reproduced.

The reporter is also indebted to the individual members indicated below for reports from which the attached list of species has been compiled. Members reporting are individually identified as follows: [A] = Dr. C. J. Alexopoulos; [B] = Dr. E. S. Beneke; [H] = Dr. L. K. Henry, Jr. (Specimens deposited in the Carnegie Musum); [O] = Mrs. Marie Overholts (Specimens in Carnegie Museum); [P] = Dr. C. L. Fergus (Specimens collected by J. W. Sinden, E. T. Reese, L. R. Kneebone and/or C. L. Fergus and deposited in the herbarium of Pennsylvania State College); [R] = Dr. C. T. Rogerson; and [S] = Dr. Walter S. Snell.

LIST OF SPECIES REPORTED

Myxomycetes: *Diderma testaceum* (Schrad.) Pers. [A & B]; *Didymium Iridis* (Ditmar) Fr. On *Sphagnum* [R3185]; *Physarum globuliferum* (Bull.) Pers. [A & B]; *Stemonitis fusca* Rost. [A & B]; *Stemonitis Smithii* Macbr. [A & B].

Phycomycetes: *Endogone pisiformis* Link. On *Sphagnum* [P; R3180; S]; *Sporodinia grandis* Link. On decaying agaric [R3183].

Ascomycetes: *Ceratostomella ulmi* (Schwarz) Buisman. On *Ulmus Americana* [P]; *Chlorosplenium aeruginosum* (Oeder.) deN. [H]; *Diatrype stigma* (Hoff.) Fr. On dead hardwood [P]; *Dibotryon morbosum* (Schw.) Th. & Syd. On *Prunus* sp. [P]; *Helvella elastica* Bull. [H]; *Endothia parasitica* (Murr.) And. & And. On *Castanea dentata* [P]; *Geoglossum glabrum* (Pers.) Fr. In *Sphagnum* [R3178]; *Guignardia Bidwellii* (Ell.) V & R. On *Vitis* sp. [P]; *Hypocrea gelatinosa* (Tode) Fr. On decaying wood [P]; *Hypocrea rufa* (Pers.) Fr. On *Diatrype stigma* [R3189]; *Hypomyces lactifluorum* (Schw.) Tul. On *Lactarius* [P]. On agaric [R3190; R3191]; *Hypomyces viridis* (A & S) Berk. & Br. On the hymenium of *Russula* sp. [R3188]; *Hypomyces violacea* (Schm.) Tul. On a myxomycete in *Sphagnum* [R3187]; *Hypoxylon coccineum* (Bull.) Fr. On dead *Fagus grandifolia* [R3175]; *Lasiosphaeria newfieldiana* Ellis & Ev. On fallen leaves in damp depression [R3177]; *Leotia lubrica* (Scop.) Pers. [P]. On mossy log [R3182]; *Leotia viscosa* Fr. On rotten log [R3181]; *Nectria cinnabarina* (Tode) Fr. On *Acer saccharum* [P]; *Nectria episphaeria* (Tode) Fr. On *Diatrype stigma* [R3193]; *Ophionectria cerea* (Berk. & Curt.) Ellis & Ev. On *Diatrype stigma* [R3194]; *Patella scutellata* (L.) Morgan [P]; *Phacobulgaria inquinans* (Fr.) Nannfeldt [H]. On *Quercus borealis* [P]; *Trichoglossum hirsutum* (Pers.) Boud. [H].

Basidiomycetes (except for Polyporaceae, Boletaceae and Agaricaceae—see below): *Calocera cornea* (Batsch.) Fr. On *Tsuga canadensis* [P]; *Calyptospora columnaris* Kuhn. On *Vaccinium corymbosum* L. [Orton]; *Clavaria cinerea* (Bull.) Fr. In coniferous woods [R3174]; *Clavaria cristata* (Holmsk.) Pers. [H]; *Clavaria fusiformis* Sowerby [H]; *Clavaria pyxidata* Pers. [O det. H]; *Dacrymyces palmatus* (Schw.) Bres. [S]; *Elaphomyces asperulus* Vitt. [Dodge]; *Hymenochaete agglutinans* Ellis [P]; *Irpex cinnamomeus* Fr. On dead hardwood [P]; *Scleroderma aurantium* (Vaill.) Pers. [H]; *Sebacina incrustans* (Pers.) Tul. Incrusting herbaceous plant [P]; *Septobasidium pinicola* Snell [S]; *Steccherinum septentrionale* (Fr.) Banker [H]; *Stereum fasciatum* Schw. On *Betula* [P]; *Stereum gausapatum* Fr. [P]; *Stereum rameale* Schw. [P; H]; *Thelephora anthocephala* (Bull.) Fr. On ground, coniferous woods [R3172]; *Thelephora multipartita* (Schw.) Fr. On ground, mixed woods [R3173]; *Thelephora palmata* Schw. [H]; *Uredinopsis mirabilis* (Peck) Magn. H. On *Onoclea sensibilis* [R3171]; *Uredinopsis osmundae* Magn. H. On *Osmunda* [Orton].

Polyporaceae: *Daedalea confragosa* (Bolt.) Fr. On *Betula* [P; H]; *Daedalea Quercina* (L.) Fr. On *Quercus* [P]; *Daedalea unicolor* (Bull.) Fr. [H]; *Favolus canadensis* Klot. [H]; *Fomes applanatus* (Pers.) Wallr. [H]. On *Acer saccharum* [P]; *Fomes fomentarius* (L.) Gill. [H]. On *Betula* [P]; *Fomes subroseus* (Weir) Overh. [H]; *Lenzites saepiaria* (Wulf.) Fr. On wood [P]; *Polyporus abietinus* (Dicks.) Fr. [H]; *Polyporus adustus* (Willd.) Fr. [H]; *Polyporus albellus* Peck. On *Betula* [P]; *Polyporus betulinus* (Bull.) Fr. [H]. On *Betula* [P]; *Polyporus borealis* Fr. [H]. On *Tsuga canadensis* [P]; *Polyporus elegans* (Bull.) Fr. On dead hardwood [P]; *Polyporus gilvus* (Schw.) Fr. On dead

hardwood [P]; *Polyporus nidulans* Fr. On *Betula* [P]; *Polyporus pargamenus* Fr. [H]. On dead hardwood [P]; *Polyporus semipileatus* Pk. [H]; *Polyporus semisupinus* Berk. & Curt. [H]; *Polyporus sulphureus* (Bull.) Fr. [H]; *Polyporus Tsugae* (Murr.) Overh. [H]; *Polyporus Tulipiferus* (Schw.) Overh. [H]; *Polyporus versicolor* (L.) Fr. [H]. On dead hardwood [P].

Boletaceae: *Boletinus pictus* (Pk.) Pk. [H; S]; *Boletus miniato-olivaceus* Frost [S]; *Boletus pallidus* Frost [S]; *Leccinum scabrum* (Bull.) S. F. Gray [S; O det. H]; *Leccinum subglabripes* (Pk.) Singer [S; H]; *Porphyrellus gracilis* (Pk.) Singer [S]; *Pulveroboletus retipes* (Berk. & Curt.) Singer [S; H]; *Suillus granulatus* (L.) Kunze [H]; *Suillus granulatus* (L.) Kunze ssp. *Snellii* Singer [S]; *Suillus subaureus* (Pk.) Snell [S]; *Tylopilus eximius* (Pk.) Singer [S].

Agaricaceae: *Amanita flavoconia* Atk. [H]; *Amanita mappa* Fr. form "A" [O det. H]; *Amanita rubescens* Fr. [H]; *Amanita virosa* Fr. [H]; *Amanitopsis vaginata* (Bull.) Roze var. *fulva* (Schaeff.) Sacc. [H; P]; *Amanitopsis vaginata* (Bull.) Roze var. *livida* Pk. [H]; *Cantharellus aurantiacus* Fr. [H; P]; *Cantharellus cinnabarinus* Schw. [H]; *Clitocybe ectypoides* Pk. [H]; *Clitocybe laccata* (Scop.) Fr. [H]; *Clitocybe laccata* (Scop.) Fr. var. *striatula* Pk. [H]; *Clitocybe odora* (Bull.) Fr. [O det. H]; *Collybia platyphylla* Fr. [H]; *Collybia radicata* (Relh.) Fr. var. *furfuracea* Pk. [H]; *Cortinarius armillatus* Fr. [H]; *Craterellus cornucopoides* (L.) Fr. [P]. On moss covered log [R3179]; *Crepidotus malachius* Berk. & Curt. [H]; *Entoloma strictus* Pk. [H]; *Galera sphagnorum* Sacc. [H]; *Hygrophorus Cantharellus* Fr. [H]; *Hygrophorus lactus* Fr. [H]; *Hygrophorus marginatus* Pk. [H]; *Hygrophorus miniatus* Fr. [H]; *Inocybe repanda* (Bull.) Quél. [H]; *Lactarius camphoratus* (Bull.) Fr. [O det. H]; *Lactarius deceptivus* Pk. [H; S]; *Lactarius griseus* Pk. [O det. H]; *Lactarius lignyotus* Fr. [H]; *Lactarius subdulcis* Pk. [H]; *Lactarius volemus* Fr. [H]; *Nyctalis asterophora* Fr. On blackened agaric [R3192]; *Panus strigosus* Berk. & Curt. [H]; *Panus stypticus* (Bull.) Fr. On wood [P]; *Paxillus atrotomentosus* Fr. [H]; *Paxillus panuoides* Fr. [S]; *Pholiota Johnsoniana* (Pk.) Atk. [H]; *Pholiota squarrosoides* Pk. [H]; *Russula alutacea* Fr. [H]; *Russula compacta* Frost & Pk. [S]; *Russula decolorans* Fr. [H]; *Russula foetens* (Pers.) Fr. [H]; *Russula fragilis* Fr. [H]; *Russula Mariae* Pk. [O det. H]; *Russula nigricans* (Bull.) Fr. [S]; *Russula xerampelina* Fr. [H].

Fungi Imperfecti: *Amblyosporium botrytis* Fresen. On *Lactarius* [P]; *Gibellula pleiopus* (Vuill.) Mains. On spider [A & R3186]; *Isaria arachnophila* Ditm. On spider in web [P]; *Septoria Lobeliae* Pk. On leaves of *Lobelia cardinalis* [R3170].

The thanks of the society were conveyed by all members present to Dr. W. Lawrence White, foray charman, and to Dr. and Mrs. Hugh M. Raup for planning this foray and for the excellent supervision and assistance they rendered to all members of our group. —G. T. JOHNSON, Department of Botany and Bacteriology, University of Arkansas, Fayetteville, Arkansas.

REVIEWS

ERWIN FRINK SMITH. A STORY OF AMERICAN PLANT PATHOLOGY, by Andrew Denny Rodgers III. x + 675 pp., 4 portraits. American Philosophical Society, Philadelphia 1952. Price, $5.00.

This biography of one of the first American plant pathologists should be of interest to anyone working in pathology or related fields. It presents not only a detailed account of the life and ideas of Smith himself, but also includes quite detailed commentaries concerning many other prominent men in various professions with whom he came in contact. His work with bacterial plant diseases is discussed in great detail, and much emphasis is placed upon his interest in human disease problems.

The author had access to Smith's diaries and papers and hence shows an intimate insight into the character of the man. This unfortunately emphasizes Smith's isolation from other plant pathologists, since many of the letters and notes are from workers in the medical field. Consequently, the book does not give a competent picture of plant pathology at the time at which Smith was active.

The frequent interruptions to discuss another person at great length and the extreme attention to detail may be disconcerting to some readers. It is, however, a record of an era in the development of plant pathology that should be familiar to present-day workers.—LOIS H. TIFFANY.

AN INTRODUCTION TO THE TAXONOMY AND NOMENCLATURE OF THE FUNGI, by G. R. Bisby. Second edition, vii + 143 pp. Commonwealth Mycological Institute, Kew, Surrey, 1953. Price 10 shillings ($1.40).

The first edition of this book achieved a success which cannot be regarded as remarkable considering the surprising amount of information compressed into a little over a hundred pages. The

exhaustion of the first edition and the substantial number of changes in the rules made at Stockholm have made a revision desirable and welcome.

The first part, Introduction and ten chapters on taxonomy, has been reprinted with little change from the first edition. The second part, Notes on Classification, is entirely new and includes a chapter on Species and Other Concepts, and one each on the five major groups: Myxothallophyta, Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. Part III, on Nomenclature, has the same chapter headings as Part II of the earlier edition, with the new Code, as revised at Stockholm, substituted for the earlier Rules, and the very useful examples of application of the articles and recommendations drawn from the fungi added where this seems desirable.

There is no work to be had for a comparable price which will be more useful to a young mycologist and there are few experienced students of the group who cannot read this admirable discussion with profit.

INDEX OF PLANT DISEASES IN THE UNITED STATES. PART V. PINACEAE—ZYGOPHYLLACEAE. INDEX. Compiled by Freeman Weiss and Muriel J. O'Brien. Plant Disease Survey, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Dept. of Agriculture. pp. 809–1263 (multigraphed). Washington. 1953.

With the completion of the last part of this monumental undertaking there is now available to mycologists a complete and up-to-date host index to parasitic fungi. For the convenience of plant pathologists, for whom, of course, the work is primarily intended bacterial, virus and nematode diseases and various others are included. The index is in three parts: to Latin host names, to common names and to others.

It is the intention of the Survey to issue Supplements "as often as occasion demands and time permits." With that promise, a vary great need has been met and mycologists will find the Index constantly useful.

MANUSCRIPT

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